STUDIES ON BIOLOGICALLY ACTIVE HETEROCYCLIC ANALOGOUS

A THESIS

SUBMITTED TO THE

SAURASHTRA UNIVERSITY

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IN

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BY

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UNDER THE GUIDANCE OF

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DEPARTMENT OF CHEMISTRY

MAHARAJA SHREE MAHENDRASINHJI SCIENCE COLLEGE

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The work included in the thesis is my own work under the supervision of **Dr. J. M. Parmar** and leads to some contribution in chemistry subsidized by a number of references.

Date : **(Niravkumar K. Joshi)**

Place: Morbi

This is to certify that the present work submitted for the Ph.D. Degree of Saurashtra University by **Niravkumar K. Joshi** (Reg.No.: 4155, Date:28/02/2009) is his own work and leads to advancement in the knowledge of chemistry. The thesis has been prepared under my supervision.

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Dedicated To My Family

ACKNOWLEDGEMENT

It is a matter of immense pleasure and proud priviledge for me to express my deep sense of gratitude to all those personality who have been helping me in diversified ways. It is a moment of gratification and pride to look back with a sense of contentment at the long traveled path, to be able to recapture some of the fine moments, to be think of the infinite number of people, some who were with me from the beginning, some who joined me at different stages during this journey, whose kindness, love and blessings has brought me to this day. I wish to thank each of them from the bottom of my heart.

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Nirav K. Joshi

CONTENTS

Synopsis

STUDIES ON BIOLOGICALLY ACTIVE HETEROCYCLIC ANALOGOUS

The work to be presented in the thesis entitled **"STUDIES ON BIOLOGICALLY ACTIVE HETEROCYCLIC ANALOGOUS"** is divided into seven chapters.

CHAPTER 1 STUDIES ON TETRAHYDROPYRIMIDINES BY CONVENTIONAL METHOD AND MICROWAVE ASSISTED METHOD

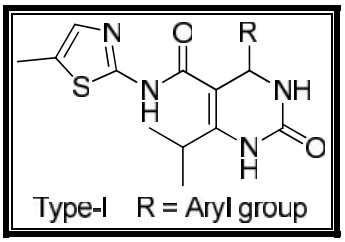
Pyrimidine nucleus possesses remarkable pharmaceutical importance and biological activities, some of their derivatives occur as natural products, like nucleic acids and vitamin B. Several pyrimidine derivatives have displayed diverse pharmacological activities like antitumor, calcium channel blocker etc. Pyrimidine moiety has attained great interest of medicinal chemists because of its broad activity spectrum. A large number of tetrahydropyrimidine derivatives can be synthesized by applying structural modification of the building blocks of Biginelli reaction. Such structural modifications have lead to interesting exhibition of biological activity. Our aim has to synthesize several modified tetrahydropyrimidines by conventional methods as well as microwave assisted synthesis.

In the present chapter, efforts have been made for the synthesis of 4-aryl-6-isopropyl-*N*-(5-methyl-1,3-thiazol-2-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (type-I) and 4-aryl-6-isopropyl-*N*-(5-methyl-1,3-thiazol-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (type-II) derivatives. 4-methyl-*N*-(5-methyl-1,3-thiazol-2-yl)-3-oxopentanamide, urea / thiourea and various aromatic aldehydes have been used to the multicomponent cyclocondensation reaction. These reactions were conventionally carried out in ethanol in the presence of the catalytic amount of con. HCl for 12-24 hours at reflux temperature. Intermediate 4-methyl-*N*-(5-methyl-1,3-thiazol-2-yl)-3-oxopentanamide was prepared by condensatation of methyl-4-methyl-3-oxopentanoate with 5-methylthiazole-2-amine .

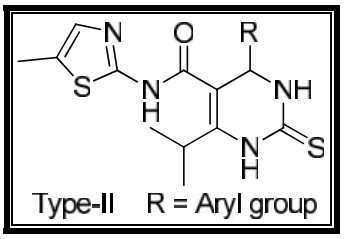
In continuation of work, the same molecules (type-I) and (type-II) when synthesized through green chemistry approach by using etidronic acid as a catalyst

through microwave irradiation technique, resulted in formation of all newly synthesized compounds with higher yields and reaction hours are also reduced to a great extent in compare to conventional method. Synthesized compounds are well characterized by elemental analysis, mass, IR and ^{1}H NMR spectroscopy.

Section-I: Synthesis and biological screening of 4-aryl-6-isopropyl-*N*-(5-methyl-1,3-thiazol-2-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide



Section-II: Synthesis and biological screening of 4-aryl-6-isopropyl-*N*-(5-methyl-1,3-thiazol-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide

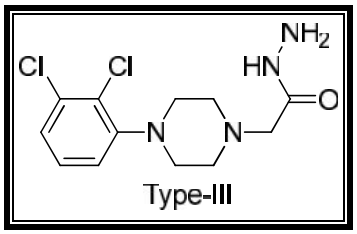


CHAPTER 2 STUDIES ON PIPERAZINE & CHROMENE DERIVATIVES

Part-1 STUDIES ON PIPERAZINE DERIVATIVES

Piperazines and their analogs are amongst the most important backbones in recent drug discovery. Moreover, piperazine derivatives are core fragments in number of API and piperazine scaffold occurs regularly in variety of complex natural products. Piperazine derivatives containing a wide spectrum of biological activities viz. antidepressant, antifungal, anti-inflammatory, antibacterial, antimalarial, anticonvulsant, antipyretic, antitumor, anthelmintics, analgesic, antitubercular, anticancer, antidiabetic etc. This observation stimulated our interest in construction, characterisation and biological evaluation of some piperazine derivatives.

Section-I: Synthesis and characterization of *2*-(4-(2,3-dichlorophenyl) piperazin-1-yl)acetohydrazide



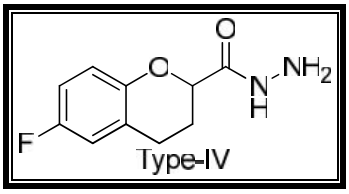
Type-III

Acetohydrazide derivative of (Type-III) has been synthesized by the condensation of ethyl 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetate with hydrazine hydrate in absolute ethanol. Intermediate ethyl 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetate was prepared by condensation of 1-(2,3-dichlorophenyl)piperazine with ethylbromoacetate.

Part-2 STUDIES ON CHROMENE DERIVATIVES

Chromene derivatives are well-known heterocyclic compounds among the organic and medicinal chemists. The synthesis of these heterocycles has received considerable attention in recent years. The derivatives of Chromene scaffold have occupied an unique place in the field of medicinal chemistry due to wide range of biological activities like antifungal, antihypertensive, anti-coagulant, antibacterial etc. All these facts were driving force to develop some novel Chromene derivatives with wide structural variation. In continuation to the same research topic, we report here some 2-substituted-3,4-dihydro-2*H*-Chromene derivatives.

Section-I: Synthesis and characterization of 6-fluoro-3,4-dihydro-2H-chromene-2-carbohydrazide



Type-IV

Carbohydrazide derivative of (Type-IV) has been synthesized by the condensation of methyl 6-fluoro-3,4-dihydro-2*H*-chromene-2-carboxylate with hydrazine hydrate in absolute ethanol. Intermediate methyl 6-fluoro-3,4-dihydro-2*H*-chromene-2-carboxylate was prepared by esterification of 6-fluoro-3,4-dihydro-2*H*-chromene-2-carboxylic acid in methanolic HCl.

CHAPTER 3 STUDIES ON SCHIFF BASE DERIVATIVES

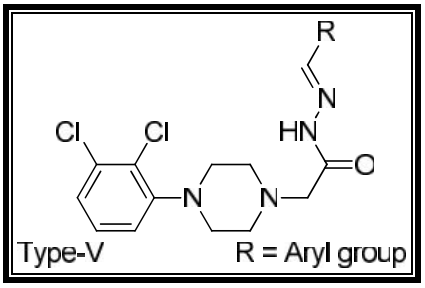
Schiff base derivatives represent one of the important classes of biological active agent which has been deeply studied during searching of new potential agent. These have been reported to be active as antimicrobial, anti-inflammatory, antitubercular, anticancer, anthelmintics etc.

Schiff bases are also known as Azomethines and they are well known intermediate for the preparation of azetidinone, thiazolidinone and many other scaffold derivatives. These are the compounds contain characteristic -C=N group, which is significantly efficient for cyclization.

In view of these observations, we have synthesized some new Schiff base derivatives, which have been described as under.

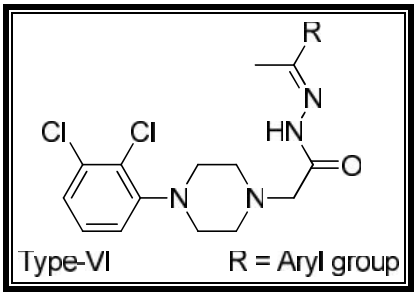
Section-I: Synthesis and biological screening of *N*'-arylmethylidene-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide

Schiff base derivatives of (Type-V) have been synthesized by the condensation of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide with various aromatic aldehydes in the presence of catalytic amount of glacial acetic acid in absolute ethanol.



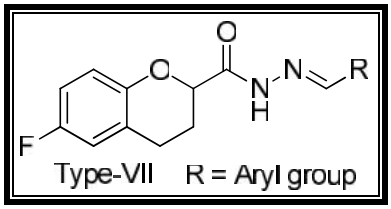
Section-II: Synthesis and biological screening of *N*'-(1-arylethylidene)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide

Schiff base derivatives of (Type-VI) have been synthesized by the condensation of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide with various aromatic acetophenones in the presence of catalytic amount of glacial acetic acid in absolute ethanol at reflux temperature.



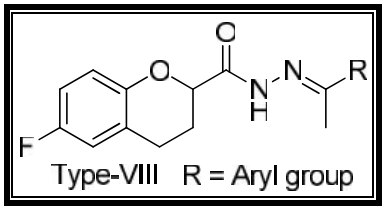
Section-III: Synthesis and biological screening of *N*'-arylmethylidene-6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide

Schiff base of (Type-VII) have been synthesized by the condensation of 6-fluoro-3,4-dihydro-2H-chromene-2-carbohydrazide with various aromatic aldehydes in the presence of catalytic amount of glacial acetic acid in absolute ethanol.



Section-IV: Synthesis and biological screening of *N*'-(1-arylethylidene)-6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrzide

Schiff base of (Type-VIII) have been synthesized by the condensation of 6-fluoro-3,4-dihydro-2H-chromene-2-carbohydrazide with various aromatic acetophenones in the presence of catalytic amount of glacial acetic acid in absolute ethanol at reflux temperature.



CHAPTER 4 STUDIES ON THIAZOLIDINONE DERIVATIVES

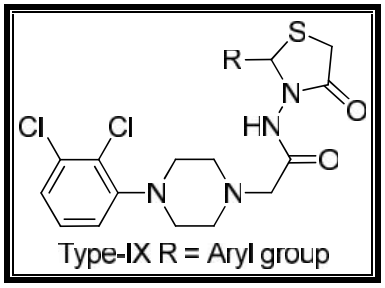
4-Thiazolidinones play a vital role due to their wide range of biological activities and industrial importance. 4-Thiazolidinones are always being an attraction point for researchers because of their efficiency exhibit by various pharmacological usages. Thiazolidinone is one of the most intensively investigated class of aromatic five

membered heterocycles. The derivatives of 4-thiazolidinone nucleus have occupied a unique place in the field of medicinal chemistry due to wide range of biological activities like antibacterial, antitubercular, anticancer, anticonvulsant and antifungal.

To approach this goal, synthesis of some new thiazolidinone derivatives have been undertaken, which have been described as under.

Section-I: Synthesis and biological screening of *N*-(2-aryl-4-oxothiazolidin-3-yl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetamide

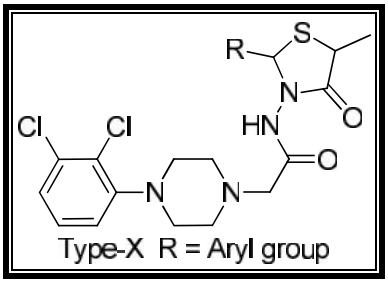
The 4-Thiazolidinone derivatives of (Type-IX) have been synthesized by the cyclocondensation reaction of Schiff base of Type (V) and thioglycolic acid.



Type-IX R = Aryl group

Section-II: Synthesis and biological screening of *N*-(2-aryl-5-methyl-4-oxothiazolidin-3-yl)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetamide

The 4-Thiazolidinone derivatives of (Type-X) have been synthesized by the cyclocondensation reaction of Schiff base of Type (V) with thiolactic acid.



Type-X R = Aryl group

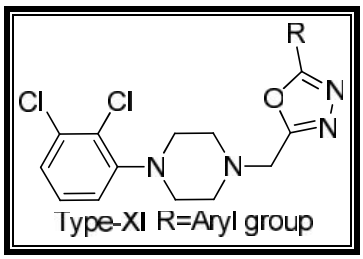
CHAPTER 5 STUDIES ON OXADIAZOLE DERIVATIVES

1,3,4- Oxadiazole derivatives constitute an important family of heterocyclic compounds. Since many of them display a remarkable biological activity and find wide usage. Particularly, the 2-aryl-5-substituted-1,3,4-oxadiazole have been reported to show broad spectrum of pharmacological activity like analgesic, anti-inflammatory, anesthetic, hypnotic, antibacterial, hypoglycemic and antifungal. These valid observations promoted

us to synthesize 1,3,4- oxadiazole derivatives with better therapeutic value, Which have been described as under.

Section-I: Synthesis and biological screening of 5-aryl-2-((4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-1,3,4-oxadiazole

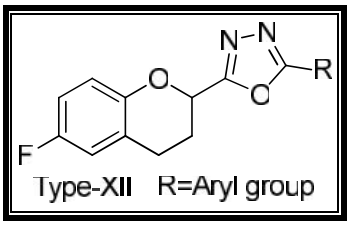
Oxadiazole derivatives of (type-XI) have been synthesized by the cyclo condensation of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide with different aromatic acids in $POCl_3$.



Type-XI R=Aryl group

Section-II: Synthesis and biological screening of 5-aryl-2-(6-fluoro-3,4-dihydro-2*H*-chromen-2-yl)-1,3,4-oxadiazole

Oxadiazole derivatives of (type-XII) have been synthesized by the cyclo condensation of 6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide with different aromatic acids in $POCl_3$.



Type-XII R=Aryl group

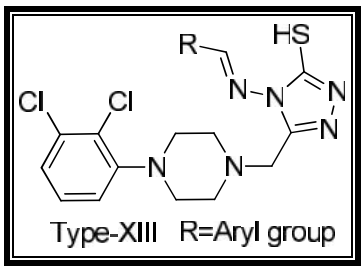
CHAPTER 6 STUDIES ON TRIAZOLE DERIVATIVES

Triazole derivatives are the important class of heterocyclic compounds, found in many potent biologically active molecules. Particularly 1,2,4-triazoles represents rapid developing field in modern heterocyclic chemistry. From literature it is predictable that, 1,2,4-triazole scaffold represents important pharmacophores, and play a vital role as medicinal agents. 1,2,4-Triazoles exhibit a wide range of biological activities such as antifungal, antibacterial, antitubercular, anticancer, analgesic and anti-inflammatory.

In view of obtaining better therapeutic agents, we have synthesized some new 1,2,4-triazole derivatives, which have been described as under.

Section-I: Synthesis and biological screening of 4-((arylmethylidine)amino)-5-((4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-4*H*-1,2,4-triazole-3-thiol

1,2,4-Triazole derivatives of (Type-XIII) have been synthesized by the condensation of 4-amino-5-((4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-4*H*-1,2,4-triazole-3-thiol with various aromatic aldehydes in the presence of catalytic amount of hydrochloric acid in absolute ethanol. Intermediate 4-amino-5-((4-(2,3-dichlorophenyl)piperazin-1-yl)methy)-4*H*-1,2,4-triazole-3-thiol has been prepared from 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide in two step.



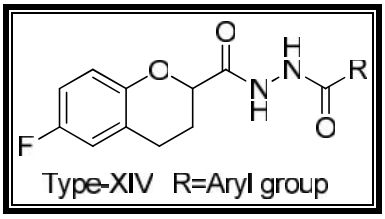
Type-XIII R=Aryl group

CHAPTER 7 STUDIES ON ARYLAMIDE DERIVATIVES

Arylamide derivatives showed different biological activity such as antihista manic, anti-inflammatory, anticonvulsant, antitubercular, antipyretic, analgesic, antiseptic etc. To getting better therapeutic agent and to evaluate its pharmacological profile, some new aryl amide derivatives have been synthesized, which have been described as under.

Section-I: Synthesis and biological screening of *N*'-(arylcarbonyl)-6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide

Aryl amides of (type-XIV) have been synthesized by the condensation of 6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide with various aromatic acids in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole (HOBt) and triethylamine in dichloromethane.



Type-XIV R=Aryl group

All the synthesized compounds have been screened for antibacterial and antifungal activity. The biological activities of the synthesized compounds have been compared with standard drugs.

The constitution of all the synthesized compounds has been characterized by using elemental analysis, ^{1}H-NMR & IR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatography.

Introduction

STUDIES ON BIOLOGICALLY ACTIVE HETEROCYCLIC ANALOGOUS

INTRODUCTION

The chemistry of the heterocyclic compounds is as logical as that of aliphatic or aromatic compounds. This study is of great interest both from the theoretical as well as practical point of view. A heterocyclic compound is one which possesses a cyclic structure with at least one different kinds of atom other than carbon in the ring system. The most common type, contains largely nitrogen, oxygen and sulphur heteroatoms, but many other elements, including even phosphorous, boron, silicon, arsenic can also serve. The heterocyclic compounds containing the less common atoms have been subject to much investigation in recent years.

The variety of heterocyclic compounds is enormous, their chemistry is complex and synthesizing them requires great skill. Among large number of heterocycles found in nature nitrogen heterocycles are most abundant than those containing oxygen or sulphur owing to their wide distribution in nucleic acid instance and involvement in almost every physiological process of plants and animals.

Heterocyclic systems are encountered in many groups of organic compounds possessing great applicability in industry as well as in our life in various ways i. e. most of the sugars and their derivatives, including vitamin C, for instance, exist largely in the form of five membered (Furanoside str.) or six membered (Pyranoside str.) ring containing one oxygen atom. Most members of the vitamin B group possess heterocyclic rings containing nitrogen; one example is vitamin B6 (Pyridoxine), which is a derivative of the pyridine essential in amino acid metabolism. Many other examples of the importance of heterocyclic compounds in biological systems can be given.

Natural products containing heterocyclic compounds such as alkaloids and glycosides have been used since old age, as medicinal agents. Adlumine alkaloid isolated from *Adlumina,* codenine isolated from opium (0.7 to 2.5 %), one of the first alkaloids isolated in pure form from seeds of *strychnos nuxvomica* these all are the examples of naturally occurring heterocyclic compounds. Many antibiotics including penicillin, cephalosporin, norfloxacin, streptomycin etc. also contain heterocyclic ring systems. Majority of the large number of drugs being introduced in pharmacopeias in recent years are heterocyclic compounds.

Important drugs, such as metformin, pioglitazone, sulphathiazole, ibuprofen, zolpidem, fluconazole, atorvastatin, reserpine, ranitidine, omeprazole, certain of the antihistamines, barbiturates etc. are heterocyclic compounds. Many veterinary products like pyrantel and morantel are the drug of choice as broad spectrum

anthelmintics. The herbicides atrazine and simazine are well known example of heterocyclic agrochemicals. Plant pigments such as indigo, heamin and anthocyanine, chlorophyll has contributed much colour chemistry and many other heterocyclic colouring matters are in use since prehistoric times.

Heterocyclic compounds are obtainable by the following methods.

(a) Isolation from natural sources, i.e. alkaloids, amino acids, indigo dyes etc.

(b) Degradation of natural products i.e. acridine, furfural, indol, pyridine, quinoline, thiophene etc.

(c) Synthesis: Synthesis methods for obtaining heterocyclic compounds may be divided into ring closer reactions, addition reaction and replacement reaction. Cyclisation is usually accomplished by elimination of some small molecules such as water or ammonia from chain of suitable length.

Heterocyclic compounds have a great applicability as drugs because,

(i) They have a specific chemical reactivity.

(ii) They play a vital role in the metabolism of all the living cells.

The current interest in the creation of large, searchable libraries of organic compounds has captured an imagination of organic chemists and the drug discovery community. Efforts in numerous laboratories focused on the introduction of chemical diversity have been recently reviewed and pharmacologically interesting compounds have been identified for libraries of widely different compositions.

Research in the field of pharmaceutical has its most important task in the development of new and better drugs and their successful introduction into clinical practice. Central to this efforts, the search for pharmaceutical substances and preparation which are new and better than previous. In addition to these objectives, we may search for newer entities which exhibit some clear advantages over a drug already known. Such advantages may be qualitatively or quantitatively improvement in activity, a partial or total absence of undesirable side effects, lower toxicity, improved stability or decreased in cost.

In medicinal chemistry the study of relationships between the chemical structure of a particular compound or group of compounds and their effects on biological activity, which is known as SAR (structural activity relationship) study and the mechanism by which the substance influences the biological system, which is known as its mode of action both play a very important role in drug discovery.

Pharmacological activity of substance is associated with molecular structure, so medicinal chemist is focused on the choice of functional groups, main scaffold and side chain for the design of new drugs. They find or encounter a situation where a structure has adequate pharmacological activity but has an inadequate pharmacokinetic profile (i.e., absorption, distribution, metabolism and excretion). This is because pharmacology and pharmacokinetic departments in the pharmaceutical industry often do not collaborate at the early stage of drug development. It is only later, when the new compound is tested in animals or in humans, that pharmacokinetic disadvantages become obvious.

Drug action is believed to be due to the drug with enzymes, receptors and other molecules found in the biological system. The binding of a drug to the active or other sites of an enzyme usually has the effect of preventing the normal operation of that enzyme. Binding of the drug to the enzyme and number of occupied binding sites play important role in inhibiting the action of the enzyme.

The degree of drug activity is directly related to the concentration of the drug in the aqueous medium in contact with the active or receptor site. The factors affecting this concentration in a biological system can be classified in to two phases.

(I) The pharmacokinetic phase

It is concerned with the study of the parameters that control the journey of the drug from its point of administration to its point of action. It includes the absorption, distribution, metabolism and elimination of a drug.

(II) The pharmacodynamic phase

It deals with the result of the interaction of drug and body cell at the receptor site, that is, what the drug does to the body. This includes physiological and biochemical effects of drugs and their mechanism of action at macromolecular/sub cellular organ systems.

Heterocyclic chemistry and medicinal chemistry share a venerable common history. Many of the founders of heterocyclic systems had an intense interest in both the natural as well as synthetic molecules. There are two main divisions of medicinal chemistry. The first chemotherapy, concerns the treatment of infections, parasite or malignant disease by chemical agents, usually substances that show selective toxicity toward the pathogen. The other division relates to bodily dysfunctions and the agents

employed are mainly compounds that effect the functioning of enzymes, the transmission of impulses or the action of hormones on receptors.

The current interest in the creation of large, searchable libraries of organic compounds has captured an imagination of organic chemist and the drug discovery community. Efforts in numerous laboratories focused on the introduction of chemical diversity have been recently reviewed and pharmacologically interesting compounds have been identified from libraries of widely different composition.

AIMS AND OBJECTIVES

Taking in view of the applicability of heterocyclic compounds, we have undertaken the preparation of heterocycles bearing tetrahydropyrimidine, piperazine and chromene nucleus. The placements of a wide variety of substituents of these nuclei have been designed in order to evaluate the synthesized products for their pharmacological profile against several strains of bacteria and fungi.

During the course of our research work, looking to the application of heterocyclic compounds, several entities have been designed, synthesized and characterized using spectral studies. The details are as under.

- To generate several derivatives of oxo/thiotetrahydropyrimidines by conventional method and by microwave irradiation method in the presence of catalyst by using MCR (Multi Component Reaction) concepts.
- To generate several derivatives like Schiff base, Thiazolidinone, Oxadiazole, Triazole, aryl amide nucleus.
- To characterize these products for structure elucidation using various spectroscopic techniques like IR, PMR and Mass spectral studies.
- To evaluate these products for antimicrobial activity by screening against different strains of bacteria and fungi.

Chapter-1

STUDIES ON TETRAHYDROPYRIMIDINES BY CONVENTIONAL METHOD & MICROWAVE ASSISTED METHOD

INTRODUCTION

Pyrimidine is a well known heterocyclic compound, which has been subjected to a large variety of structural modification in order to synthesize derivatives with different biological properties. Pyrimidine derivatives have been reported to possess a broad spectrum of pharmacological properties.

Pyrimidine is the most important member of all the diazines as this ring system occurs widely in living organisms. Purines, uric acid, barbituric acid and some anti-malarial and anti-bacterial agents also contain the pyrimidine ring. The chemistry of pyrimidine has been widely studied. Pyrimidine was first isolated by Gabriel and Colman in 1899. Despite the importance of dihydroazines (particularly those containing the 1,4-dihydropyrimidine and dihydropyridine moiety[1]) for clarifying a wide range of theoretical, medicinal and biological problems, the chemistry of this group of compounds is still extremely spotty.[2-6]

From the theoretical point of view, it is essential to predict the structure, binding properties, chemical reactivity, etc. of dihydro compounds from the number and positioning of nitrogen atoms in the ring, as well as from the disposition of double bonds. Such quantum mechanical calculations also enable an evaluation of the degree of aromatic character in potential "homoaromatic" and "antiaromatic" isomers. Availability of novel model compounds for verifying these predictions would open up new horizons in theoretical heterocyclic chemistry, particularly in clarifying the structures leading to spontaneous isomerization of a derivative or in verifying its redox properties.

From the biochemical point of view, dihydroazines are of intense interest because of presence of this group at the active site of the hydrogen transferring coenzyme (nicotinamide adenine dinucleotied hdrogenase-NADH or reduced nicotinamide adenine dinucleotide). This nucleotide, a central participant in metabolic processes in living organisms, participates in the reduction of various unsaturated functionalities.

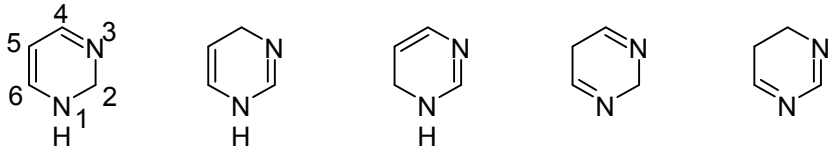
In drug development, dihydroazines show great promise, particularly since the 4-aryldihydropyridines exhibit powerful vasodilation activity via modifying the calcium ion membrane channel.[7-10] Additionally, dihydropyridines have been found to actively transport medication across biological membranes.[11]

Until recently, most of the information available on dihydroazines centered around dihydropyridines, with very little data extending to the related dihydropyrimidines.

This lacuna has motivated our deep involvement in developing dihydropyrimidine chemistry, particularly dihydropyrimidines without any substituents on the ring

nitrogen.[12] These molecules have long been considered unstable for oxidation, polymerization or disproportionation reactions.[13]

Figure below present the five possible isomeric structures of dihydropyrimidines, exhibiting different dispositions of the double bonds.



1,2- 1,4- 1,6- 2,5- 4,5-

However, these structures are not easy to synthesize and, as a result, most of the known dihydropyrimidines have either 1,2- or the tautomeric 1,4- and 1,6- geometry. On the basis of data available in the literature[14,15], the dihydropyrimidines can be conveniently divided into two groups, within each of which interconversion between isomers is possible under thermal conditions, namely, the 1,4-, 1,6-, and 4,5- isomers, and the 1,2- and 2,5- isomers. It is worthwhile to note that, while thermal interconversion between the two groups is not observed, photochemical rearrangement of 1,4- (or 1,6-) dihydropyrimidines to 1,2-isomers has been reported[16,17].

It should be stressed that dihydroazines take part in various isomerization processes, usually characterized by reversible or irreversible migrations within the ring, the study of which is still in its infancy. Hydrogen migration, for example, is classified either as rearrangement or tautomerism depending on its kinetic and thermodynamic parameters, the former term is reserved for irreversible processes, while the latter is used to describe fast reversible exchanges[18]. A study of isomerization in dihydropyrimidines provides an excellent opportunity for clarifying the factors regulating these processes.

After successfully developing versatile synthetic techniques for obtaining a variety of 1,4- and 1,6-dihydropyrimidines[19-21], as well as the observation of amidinic tautomerism between the two[22,23], A. L. Weis et al.[14] examining the possibility of preparative synthesis of similarly 1,2-dihydro derivatives and studying their properties. Particularly important goals of this study were the possible observation of the formally allowed hydrogen shift[23], of homoaromaticity[24,25] or of imine-enamine tautomerism[26] in these compounds, behaviors of which have been seen in other systems.

Recently few reports on the formation of 1,2-dihydropyrimidines exist in the literature, and in those cases where a product could be isolated and characterized, the material was either an *N*-substituted derivative or else it contained geminal disubstitution

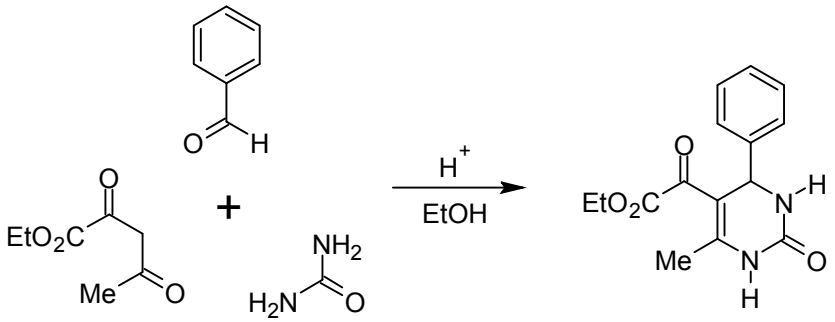
at position 2, situations that prevent the molecule from oxidizing to the corresponding pyrimidine.

Pyrimidine ring carrying various substituents may be built up from two or three small fragments by the principle synthesis or by a variety of other synthesis, which are complimentary rather than alternative to it. A second type of synthesis is the isomerisation or break down of another heterocycles such as hydration of purine but such roots are frequently used.

SYNTHETIC ASPECT

Biginelli Reaction

In 1893, Italian chemist Pietro Biginelli[27] reported an acid catalyzed cyclocondensation reaction of ethyl acetoacetate, benzaldehyde, and urea. The reaction was carried out by simply heating a mixture of the three components dissolved in ethanol with a catalytic amount of HCl at reflux temperature. The product of this novel one-pot, three-component synthesis that precipitated on cooling of the reaction mixture was identified correctly by Biginelli as 3,4-dihydropyrimidin-2(1*H*)-one.



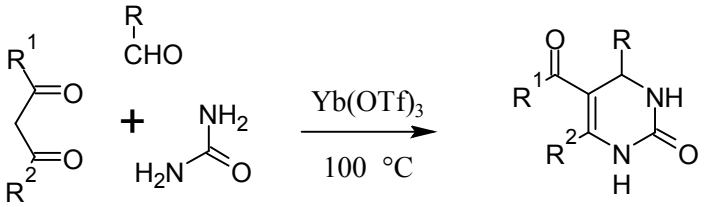
Alternative synthetic routes for better yield, shorter reaction time to synthesize new analogs

Various modifications have been applied to Biginelli reaction to get better yield and to synthesize biologically active analogs. Different catalysts have been reported to increase the yield of the reaction. Microwave synthesis strategies have also applied to shorten the reaction time. Solid phase synthesis and combinatorial chemistry has made possible to generate library of DHPM analogs. The various modifications are discussed in the following section.

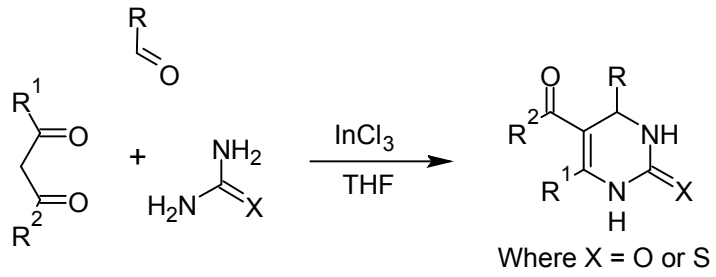
Catalysts

Min Yang and coworkers[28] have synthesized the different DHPMs by using different inorganic salts as a catalyst. They found that the yields of the one-pot Biginelli reaction can be increased from 20-50 % to 81-99 %, while the reaction time shorted for 18-24 hr

to 20-30 min. This report discloses a new and simple modification of the Biginelli type reaction by using $Yb(OTf)_3$ and $YbCl_3$ as a catalyst under solvent free conditions. One additional important feature of this protocol is the catalyst can be easily recovered and reused.

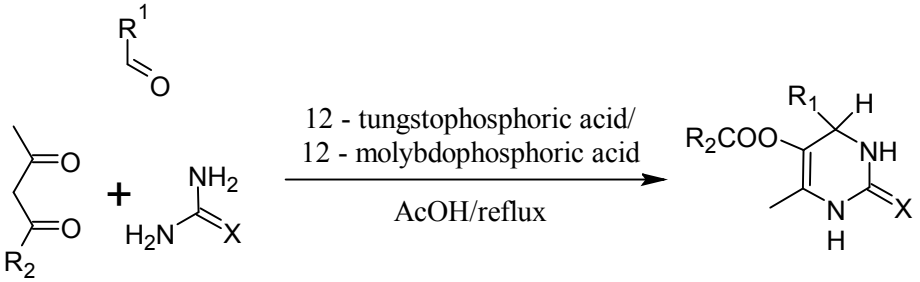


Indium(III) chloride was emerged as a powerful lewis catalyst imparting high region and chemo selectivity in various chemical transformations. B. C. Ranu and coworkers[29] reported indium(III) chloride ($InCl_3$) as an efficient catalyst for synthesis of 3,4-dihydropyrimidn-2(1*H*)-ones. A variety of substituted aromatic, aliphatic and heterocyclic aldehydes have been subjected to this condensation very efficiently. Thiourea has been used with similar success to provide the corresponding dihydropyrimidin-2(1*H*)-thiones.



Where X = O or S

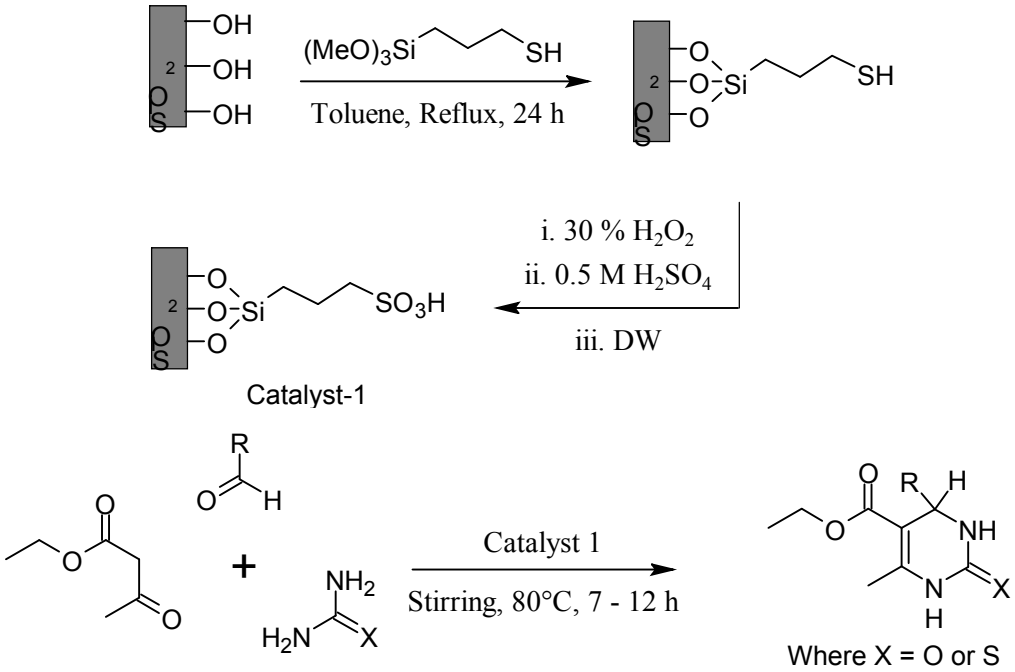
Majid M. Heravi et al. have reported a simple, efficient and cost-effective method for the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones/thiones by one pot three-component cyclocondensation reaction of a 1,3-dicarbonyl compound, an aldehyde and urea or thiourea using 12-tungstophosphoric acid[30] and 12-molybdophosphoric acid[31] as a recyclable catalyst.



Where X = O or S

A novel covalently anchored sulfonic acid onto the surface of silica was prepared and investigated for the Biginelli reaction by Satya Paul and co-workers[32]. The catalyst is

highly stable, completely heterogeneous and recyclable for several times. The workup procedure is very simple and products were obtained in good to excellent yields.



An efficient three-component synthesis of 3,4-dihydropyrimidinones using trichloroisocyanuric acid (TCCA) as mild, homogeneous and neutral catalyst for Biginelli reaction in ethanol or DMF under reflux condition[33].

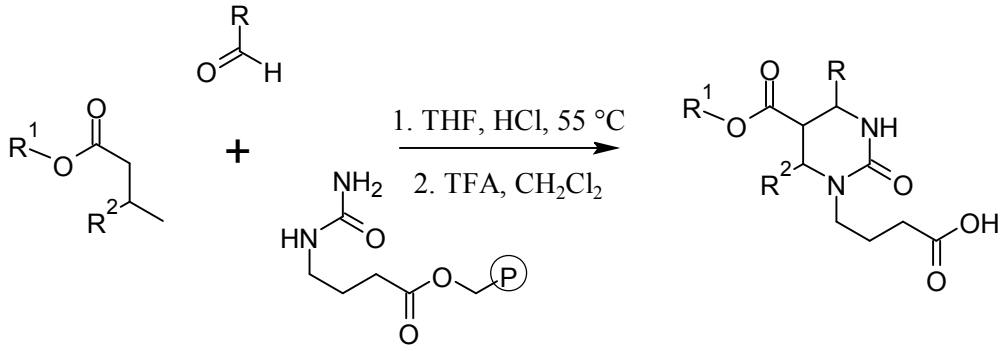
Very recently, many researchers[34-40] have investigated an efficient Biginelli reaction under solvent-free conditions for one-pot synthesis of 3,4-dihydropyrimidin-2-ones/thiones using various catalyst.

Solid phase synthesis

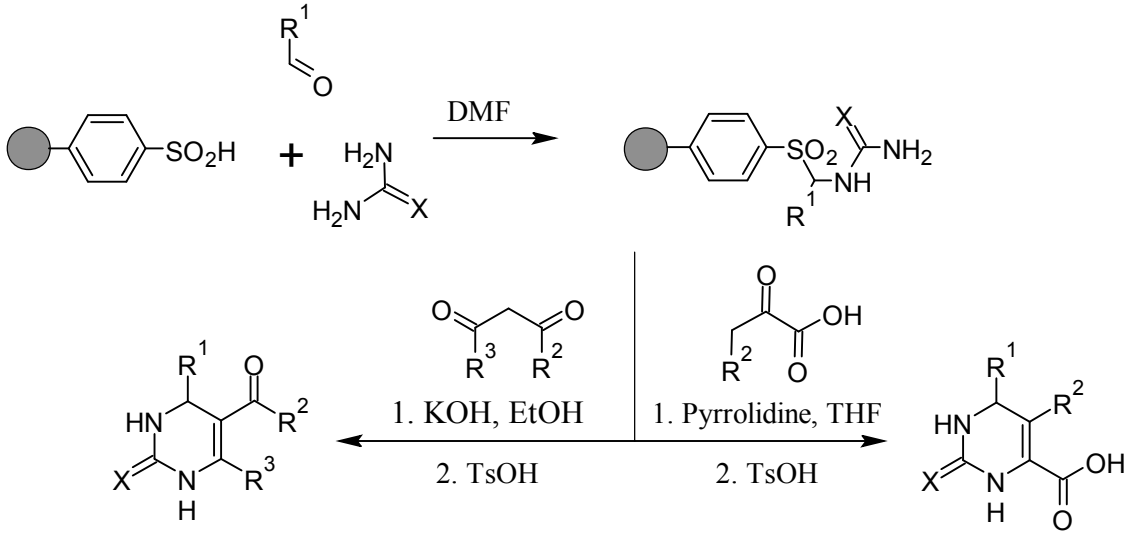
The generation of combinatorial libraries of heterocyclic compounds by solid phase synthesis is of great interest for accelerating lead discovery and lead optimization in pharmaceutical research[41,42]. Multi-component reactions (MCRs) leading to heterocycles are particularly useful for the creation of diverse chemical libraries, since the combination of n (where $n \geq 3$) building blocks in a single operation leads to high combinatorial efficiancy[41-43]. Therefore, solid phase modifications of MCRs are rapidly become the cornerstone of combinatorial synthesis of small-molecule libraries[41-47].

The first solid-phase modification of the Biginelli condensation was reported by Wipf and Cunningham[48] in 1995. In this sequence, γ-aminobutyric acid derived urea was attached to Wang resin using standard procedures. The resulting polymer-bound urea was condensed with excess β-ketoester and aromatic aldehydes in THF at 55 °C in the presence of a catalytic amount of HCl to afford the corresponding immobilized DHPMs.

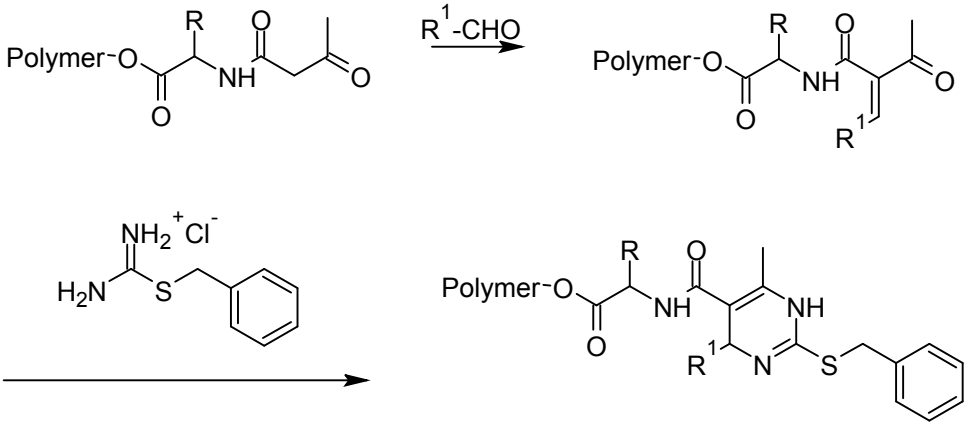
Subsequent cleavage of product from the resin by 50 % trifluoroacetic acid (TFA) provided DHPMs in high yields and excellent purity.



Weiwei Li and Yulin Lam[49] described the synthesis of 3,4-dihydropyrimidin-2-(1*H*)ones/thiones using sodium benzenesulfinate as a traceless linker. The key steps involved in the solid-phase synthetic procedure include sulfinate acidification, condensation of urea or thiourea with aldehydes and sulfinic acid and traceless product release by a one-pot cyclization-dehydration process. Since a variety of reagents can be used, the overall strategy appears to be applicable to library generation.



Recently, Gross et al.[50] developed a protocol based on immobilized α-ketoamides to increase the diversity of DHPM. The resulting synthetic protocol proved to be suitable for the preparation of a small library using different building blocks. They found that the expected DHPM derivatives were formed in high purity and yield, if aromatic aldehyde and α-ketoamide building blocks were used. The usage of an aliphatic aldehyde leads to an isomeric DHPM mixture. Purities and yields were not affected if thiourea was used instead of urea.

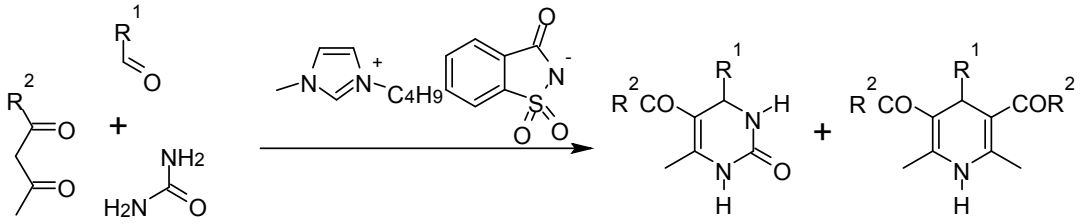


Liquid phase synthesis

In the solid phase synthesis there are some disadvantages of this methodology compared to standard solution-phase synthesis, such as difficulties to monitor reaction progress, the large excess of reagents typically used in solid-phase supported synthesis, low loading capacity and limited solubility during the reaction progress and the heterogeneous reaction condition with solid phase[51]. Recently, organic synthesis of small molecular compounds on soluble polymers, i.e. liquid phase chemistry has increasingly become attractive field[52]. It couples the advantages of homogeneous solution chemistry with those of solid phase chemistry.

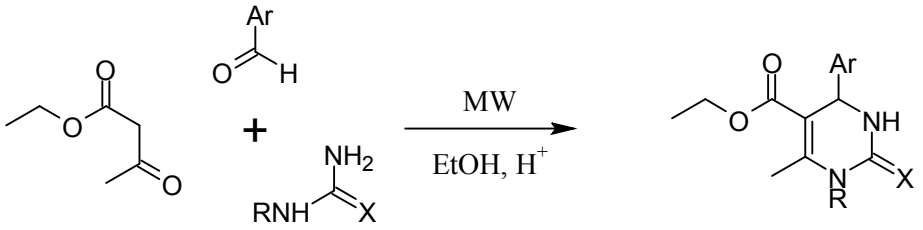
Moreover owing to the homogeneity of liquid-phase reactions, the reaction conditions can be readily shifted from solution-phase systems without large changes and the amount of excessive reagents is less than that in solid-phase reactions. In the recent years, Task Specific room temperature Ionic Liquids (TSILs) has emerged as a powerful alternative to conventional molecular organic solvents or catalysts. Liu Zuliang et al.[53] reported cheap and reusable TSILs for the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones via one-pot three component Biginelli reaction.

Ionic liquid-phase bound acetoacetate react with thiourea and various aldehydes with a cheap catalyst to afford ionic liquid-phase supported 3,4-dihydropyrimidin-2(1*H*)-thiones by Jean Pierre Bazureau and co-workers[54]. 3,4-dihydropyrimidinones was synthesized in one-pot of aldehydes, β-dicarbonyl compounds and urea, catalyzing by non-toxic ionic liquid 1-n-butyl-3-methylimidazolium saccharinate (BMImSac)[55] at room temperature.



Microwave assisted synthesis

In general, the standard procedure for the Biginelli condensation involves one pot condensation of the three building blocks in a solvent such as ethanol using a strongly acidic catalyst that is hydrochloric acid[56]. One major drawback of this procedure, apart from the long reaction times involving reflux temperatures, are the moderate yields frequently observed when using more complex building blocks. Microwave irradiation (MWI) has become an recognized tool in organic synthesis, because the rate enhancement, higher yields and often, improved selectivity with respect to conventional reaction conditions[57]. The publication by Anshu Dandia et al.[58] described microwave-enhanced solution-phase Biginelli reactions employing ethyl acetoacetate, thiourea and a wide variety of aromatic aldehydes as building blocks. Upon irradiation of the individual reaction mixtures (ethanol, catalytic HCl) in an open glass beaker inside the cavity of a domestic microwave oven the reaction times were reduced from 2-24 hours of conventional heating 80 °C, reflux to 3-11 minutes under microwave activation (ca. 200 – 300 W). At the same time the yields of DHPMs obtained were markedly improved compared to those reported earlier using conventional conditions.

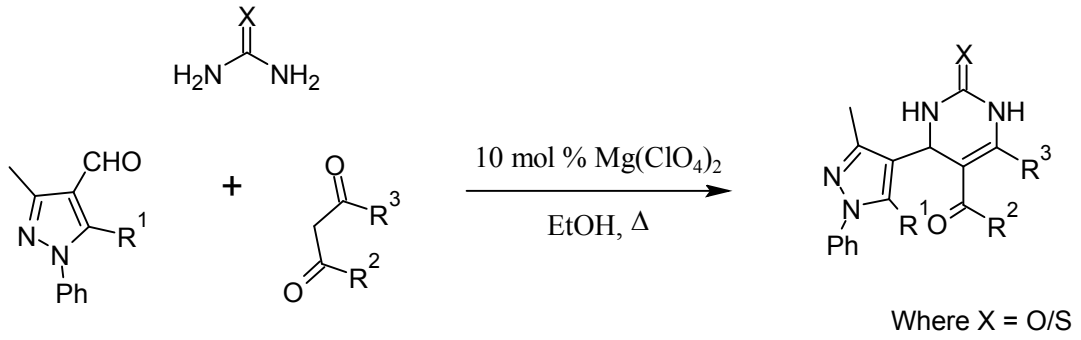


In recent years, solvent free reactions using either organic or inorganic solid supports have received increasing attention[59]. There are several advantages to performing synthesis in dry media: (i) short reaction times, (ii) increased safety, (iii) economic advantages due to the absence of solvent. In addition, solvent free MWI processes are also clean and efficient. Activated fly ash, an industrial waste (pollutant) is an efficient and novel catalyst for some selected organic reactions in solvent free conditions under microwave irradiation[60]. M. Gopalakrishnan and co-workers have reported Biginelli reaction under microwave irradiation in solvent-free conditions using activated fly ash as a catalyst.

Ultrasound assisted synthesis

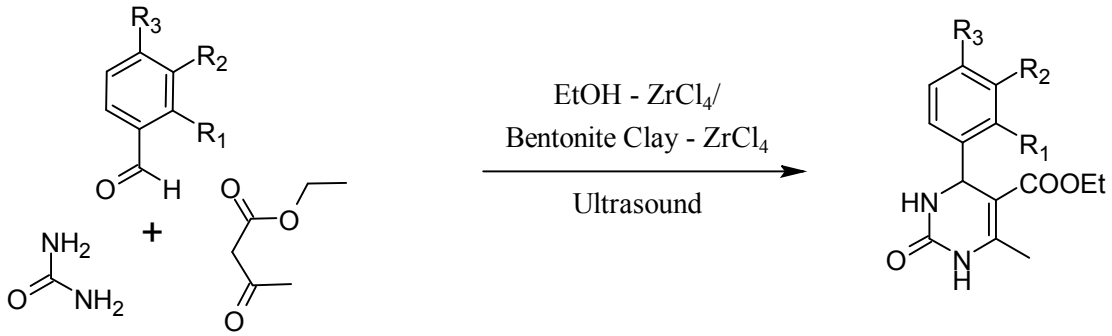
Ultrasound as a green synthetic approach has gradually been used in organic synthesis over the last three decades. Compared with the traditional methods, it is more convenient, easier to be controlled, and consumes less power. With the use of ultrasound irradiation, a large number of organic reactions can be carried out in milder conditions with shorter reaction time and higher product yields[61]. Ultrasound irradiated and amidosulfonic acid (NH_2SO_3H) catalyzed synthesis of 3,4-dihydropyrimidi-2-(1*H*)ones have reported by Ji-Taai Li and co-workers[62] using aldehydes, β-ketoester and urea.

Chenjiang Liu et al.[63] have synthesized a novel series of 4-substituted pyrazolyl-3,4-dihydropyrimidin-2(1*H*)-thiones under ultrasound irradiation using magnesium perchlorate [$Mg(ClO_4)_2$] as catalyst, by the condensation of 5-chloro/phenoxyl-3-methyl-1-phenyl-4-formylpyrazole, 1,3-dicarbonyl compound and urea or thiourea in moderate yields. The catalyst exhibited remarkable reactivity and can be recycled.



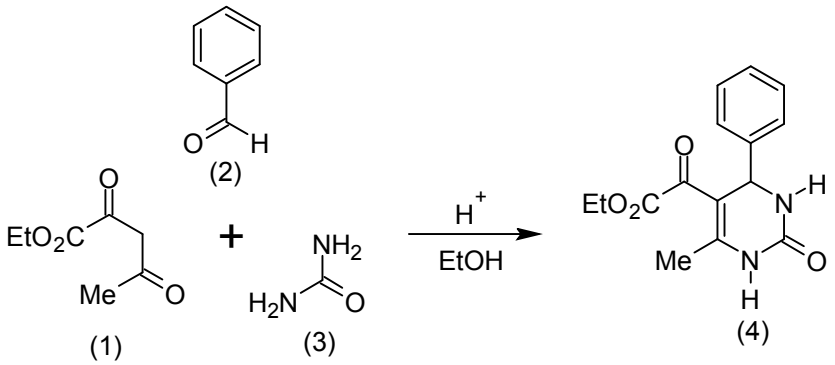
Where X = O/S

Sonication of aromatic aldehydes, urea and ethyl acetoacetate in presence of solvent (ethanol) or solvent-less dry media (bentonite clay) by supporting-zirconium chloride ($ZrCl_4$) as catalyst at 35 kHz gives 6-methyl-4-substitutedphenyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters proficiently in high yields reported by Harish Kumar et al[64].

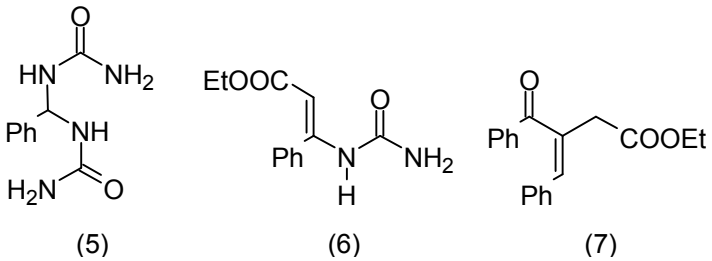


REACTION MECHANISM

In 1893 Biginelli[65] reported the first synthesis of dihydropyrimidines by a simple one-pot condensation reaction of ethyl acetoacetate, benzaldehyde and urea.

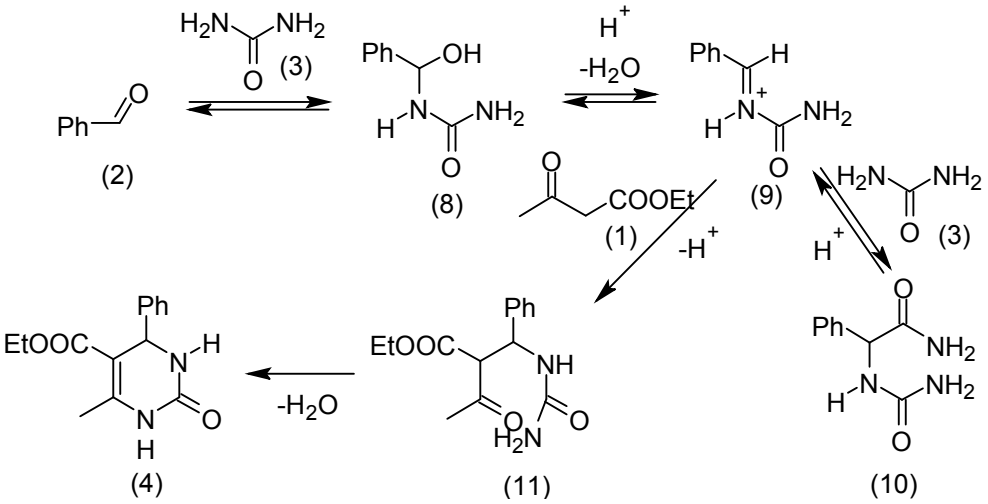


Despite the importance and current interest in dihydropyrimidines of the Biginelli type, the mechanism of the classical three-component Biginelli condensation has not been elucidated with certainty[66]. Since the 1930s several mechanistic pathways have been proposed for the Biginelli reaction. In 1933, Folkers and Johnson[67] reported that one of three intermediates was likely to be present in this reaction.



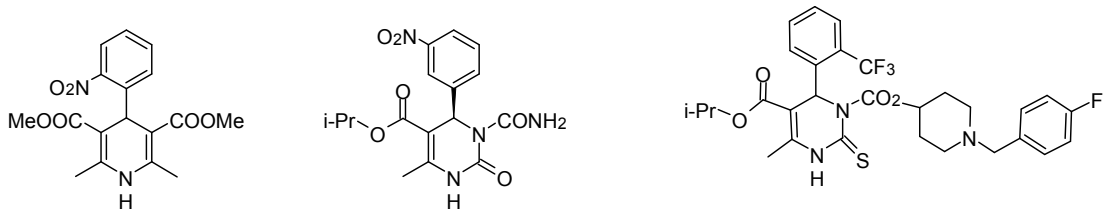
Fourty years after the initial praposal, In 1973 Sweet and Fissekis[68] proposed more detailed pathway involving carbenium ion spices, The mechanism was then reexamined 25 years later in 1997 by Kappe, C. O. Kappe[69] used ^{1}H and ^{13}C-NMR spectroscopy to support the argument that the key intermediate in the Biginelli reaction was iminium species (9). In the event, benzaldehyde (2) reacted with urea (3) to form an intermediate "hemiaminal" (8) which subsequently dehydrated to deliver N-acyl imminium spices (9). Iminium cation (9) then reacted with ethyl acetoacetate (1) to give uride (11), which underwent facile cyclodehydration to give (4). Kappe also noted that in the absence of (1), bisureide (10) was afforded as a consequence of nucleophilic attack of (9) by urea (3). This discovery confirmed the conclusion of Folkers and Johnson[67] in 1933. As far as the proposal from 25 years earlier by Sweet and Fisselus, Kappe saw no evidence by ^{1}H and ^{13}C-NMR spectroscopy that a carbenium ion was a required species

in the Biginelli reaction. The reaction mechanism can therefore be classified as α-amidoalkylation, or more specifically as α-uridoalkylation[70].



THERAPEUTIC IMPORTANCE

4-Aryl-1,4-dihydropyridines (DHPs) of the nifedipine type e.g. nifedipine are the most studied class of organic calcium channel modulators. More than 30 years after the introduction of nifedipine (12), many DHP analogs have now been synthesized and numerous second-generation commercial products have appeared on the market e.g. nitrendipine, nicardipine and amlodipine[71]. The aza-analogs such as dihydropyrimidines (13) which show a very similar pharmacological profile to classical dihydropyridine calcium channel modulators[72-76]. Over the past several lead-compounds were developed e.g. (13) SQ 32926 and (14) SQ 32574[73,75] that are superior in potency and duration of antihypertensive activity to classical dihydropyridine drugs and compare favorable with second-generation analogs such as amlodipine and nicardipine[73].

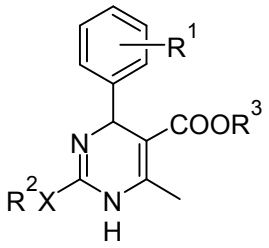


(12) (13) SQ 32926 (14) SQ 32574

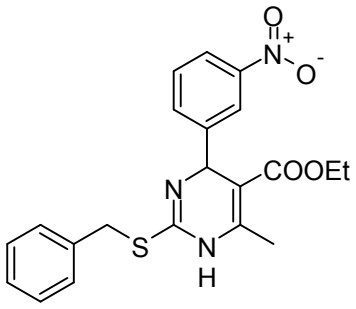
Calcium ion plays a vital role in a large number of cellular processes, including excitation-contraction and stimulus-secretion[77,78]. The regulation of the intracellular concentration of this ion makes possible the control of such Ca^{2+} dependent processes. One means of accomplishing this is by the use of agents known as calcium channel

antagonists, which inhibit the movement of calcium through certain membrane channel[79-81].

K. S. Atwal[82] prepared the 2-heterosubstituted-4-aryl-l,4-dihydro-6-methyl-5 pyrimidinecarboxylic acid esters (15), which lack the potential C_3 symmetry of dihydropyridine calcium channel blockers, were prepared and evaluated for biological activity. Biological assays using potassium-depolarized rabbit artery and radioligand binding techniques showed that some of these compounds are potent mimics of dihydropyridine calcium channel blockers. The combination of a branched ester e.g. isopropyl, sec-butyl and thioalkyl group e.g. –SMe group was found to be optimal for biological activity. Dihydropyrimidines (15) were found to be 30 fold less active than dihydropyridines. The solid-state structure of dihydropyrimidine analogue (16) shows that these compounds can adopt a molecular conformation which is similar to the reported conformation of dihydropyridine calcium channel blockers.

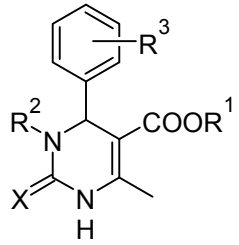


(15)

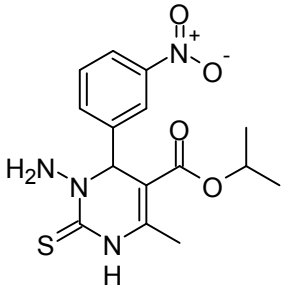


(16)

K. Atwal et al.[83] synthesized the 3-substituted 1,4-dihydropyrimidine (17) and documented that vasorelaxant activity was critically dependent on the size of the C_5 ester group, isopropyl ester being the best, a variety of substituents (carbamate, acyl, sulfonyl, alkyl) were tolerated at third position. The dihydropyrimidines (17) are significantly more potent than corresponding 2-heteroalkyl-l,4-dihydropyrimidines. Dihydropyridine enantiomer usually show 10-15 fold difference in activity, while the enantiomers of dihydropyrimidine (18) show more than a 1000 fold difference in activity. These results strengthen the requirement of an enamino ester for binding to the dihydropyridine receptor and indicate a nonspecific role for the substituent present on the third position.

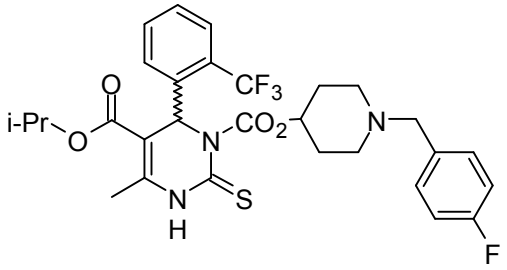


(17)



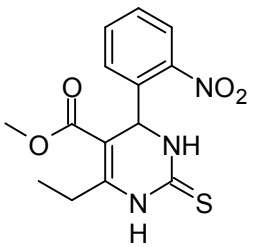
(18)

George C. Rovnyak et al.[84] examined a series of novel dihydropyrimidine calcium channel blockers that contain a basic group attached to either C_5 or N_3 of the heterocyclic ring. One of these compounds was identified as a lead, and the individual enantiomers (19a) (R) and (19b) (S) were synthesized. Dihydropyrimidine (19a) is equipotent to nifedipine and amlodipine in vitro. In the spontaneously hypertensive rat, dihydropyrimidine (19a) is more potent and longer acting than nifedipine and compares most favorably with the long-acting dihydropyridine derivative amlodipine. Dihydropyrimidine (19a) has the potential advantage of being a single enantiomer.

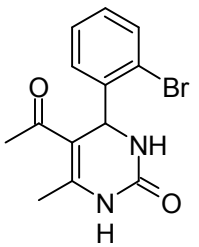


(19a) (R), (19b) (S)

Selma Sarac and co-workers[85,86] have synthesized 4-arlyl-3,4-dihydropyrimidin-2(1H)-one/thione derivatives. The calcium channel blocker activities of all compounds performed on isolated rat ileum. Product (20), 2-nitrophenyl derivative and (21), 2-bromophenyl derivative have potent antispasmodic activity on $BaCl_2$ stimulated rat ileum.



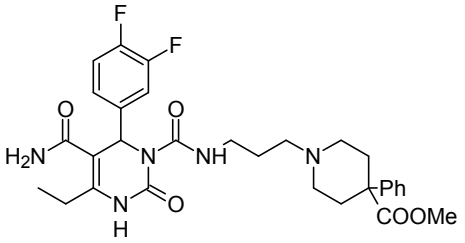
(20)



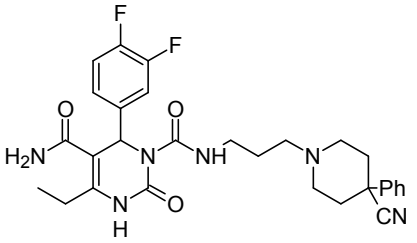
(21)

N. Dhanapalan and co-workers[87] have synthesized dihydropyrimidinones and describe compound (22) have a high binding affinity (*Ki* = 0.2nM) for α_{1a} receptor and greater than 1500 fold selectivity over α_{1b} and α_{1d} adreno receptors. Modification of the

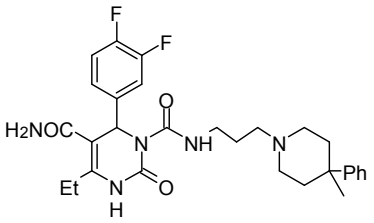
linker in (22) gave compounds (23) and (24)[88] viz μ-opioid receptor. Both these compounds showed good α_{1a} binding affinity (*Ki* = 0.2nM) and selectivity (>800-fold over α_{1b} and α_{1d}), also showed good selectivity over several other recombinant human G-protein coupled receptors. They have also identified that compound (25)[89] was a lead compound with a binding and functional profile comparable to that of (22). (25) have negligible affinity for the μ-opioid receptor.



(22)



(23)



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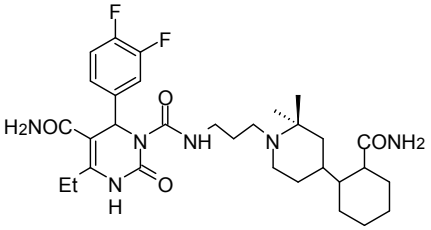
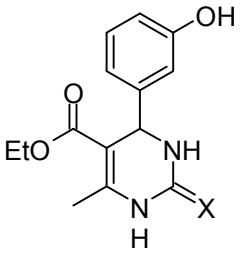


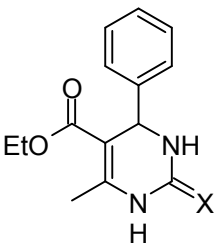
(25)

The synthesis and differential antiproliferative activity of monastrol (26a), oxomonastrol (26b) and eight oxygenated derivatives (28a,b)-(31a,b) on seven human cancer cell lines are described by Dennis Russowsky[90]. For all evaluated cell lines, monastrol (26a) was shown to be more active than its oxo-analogue, except for HT-29 cell line, suggesting the importance of the sulfur atom for the antiproliferative activity. Monastrol (26a) and the thio derivatives (28a), (29a) and (31a) displayed relevant antiproliferative properties with 3,4-methylenedioxy derivative (31a) being approximately more than 30 times more potent than monastrol (26a) against colon cancer (HT-29) cell line.



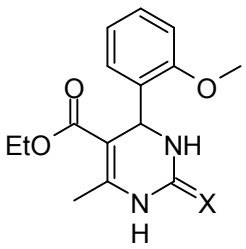
(26a) X = S Monastrol

(26b) X = O Oxo-monastrol



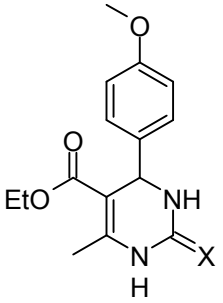
(27a) X = S

(27b) X = O



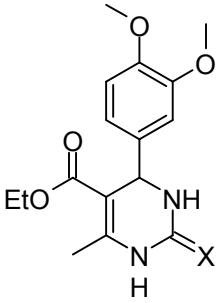
(28a) X = S

(28b) X = O



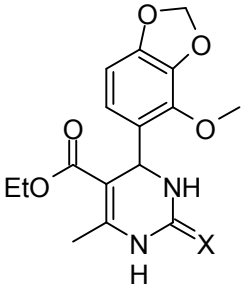
(29a) X = S

(29b) X = O



(30a) X = S

(30b) X = O



(31a) X = S

(31b) X = O

Y. Mizutani and co-workers[91,92] identify that dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme in the pathway of uracil and thymine metabolism. DPD is also the principle enzyme involved in the degradation of 5-fluorouracil and anticancer chemotherapeutic agent that is used clinically to treatment of bladder cancer and renal cell carcinoma.

CATALYTIC STUDY OF ETIDRONIC ACID THROUGH MICROWAVE IRRADIATION TECHNIQUE

More than 100 year ago the first 1,4-DHPMs were synthesized by Pietro Biginelli[93]. The classical method involves a simple one-pot condensation reaction of ethylacetoacetate, aryl aldehyde and urea/thiourea under strong acidic condition[94]. The major limitations of biginelli reaction are lower yield and longer reaction time. Moreover, product separation and work up also cause problem and needed special techniques. Thus, main disadvantages in biginelli reaction are lower yield, longer reaction time and tidious work up.

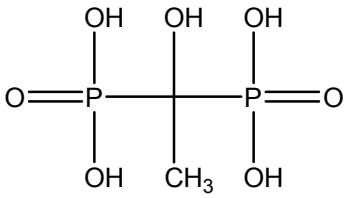
Due to these disadvantages of biginelli method, there are several efficient methods developed for the synthesis of 1,4-DHPMs, which comprise the use of Silica triflate[95], Iodotrimethylsilane in acetonitrile[96], Strontium(II)nitrate[97], PEG-4000[98], chloroacetic acid[99], $InBr_3$[100], microwave[101,102], KSF montmorillonite[103] etc as catalysts. However, the use of high temperatures, expensive metal precursors and longer reaction times are limits of these methods.

CURRENT WORK

To avoid these unconvenency, the development of an efficient and versatile method has been made for the synthesis of 1,4-DHPMs, The work of such heterocycle is an interesting research area and there is a scope for further improvement towards

conventional reaction conditions and to improve the reaction yield and decrease the reaction time.

In extension of our work, the tetrahydropyrimidine derivatives have been synthesized through green chemistry approach by utilizing Etidronic acid through microwave irradiation technique.



(44)

Etidronic acid [(1-hydroxyethylidene)bisphosphonic acid]

Etidronic acid [(1-hydroxyethylidene)bisphosphonic acid] is a phosphonic acid and is also known as a bisphosphonate having a molecular formula $C_2H_8O_7P_2$. The two PO_3 (phosphonate) groups are covalently linked to a single carbon atom.[104] By using this method we found increased yield about 20-25% more than conventional method.

COMPARISION OF CONVENTIONAL BIGINELLI METHOD AND MICROWAVE ASSISTED METHOD

The microwave assisted organic synthesis (MAOS) are attracting the interest of organic chemists and other researchers due to their significant potential for converting reactant into respective product in short reaction time with quantitative yields. Several procedures have been reported in the literature for the synthesis of DHPMs under microwave irradiations with different conditions and differnt catalyst.

In this chapter we have synthesised tetrahydropyrimidine derivatives of type-I and type-II by conventional beginelli method and also by microwave assisted method, the results obtained from the both method are noted. Comparition of the catalyst used, reaction time and yield in both methods are given below in tabular form.

Parameter	Conventional biginelli method	Microwave assisted method
Catalyst	Con. HCl	Etidroni acid
Reaction time	10 to 20 hours	6 to 18 minutes
Yield	40 to 60 %	60 to 90 %

Thus, by using microwave assisted impruved method, it get optimum yields and also reaction hours are reduced to a great extent (from hours to minutes) in comparison with conventional biginelli method as mentioned in literature.

WORK DONE FROM OUR LABORATORY

Synthesis anticancer, antitubercular and antimicrobial activity of some new pyrimidine derivatives have been reported by K. S. Nimavat[105], some new thiopyrimidine and oxopyrimidine heterocycles bearing 4-(methylsulfonyl)phenyl nucleus as potent antitubercular and antimicrobial agents was developed and reported by D. J. Paghdar[106]. M. R. Patel[107] have reported synthesis and evaluation of pharmacological activity of some new aminopyrimidine and thiopyrimidine derivatives.

J. D. Akbari and coworkers reported synthesis of some new 1,2,3,4-tetrahydropyrimidine-2-ones and their thiazolo[3,2-*a*]pyrimidine derivatives as a potential biological agents[108], synthesis of some new pyrazolo[3,4-*d*]pyrimidines and thiazolo[4,5-*d*]pyrimidines and evaluation of their antimicrobial activities[109], green chemistry approach to synthesis of some new trifluoromethyl containing tetrahydropyrimidines under solvent free conditions[110], synthesis and antimicrobial activities of some new pyrazolo[3,4-*d*]pyrimidines and thiazolo[4,5-*d*]pyrimidines[111].

SECTION-I: SYNTHESIS OF 4-ARYL-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDES USING CONVENTIONAL METHOD & MICRO-WAVE ASSISTED METHOD AND BIOLOGICAL SCREENING.

SECTION-II: SYNTHESIS OF 4-ARYL-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDES USING CONVENTIONAL METHOD & MICROWAVE ASSISTED METHOD AND BIOLOGICAL SCREENING.

SECTION-I

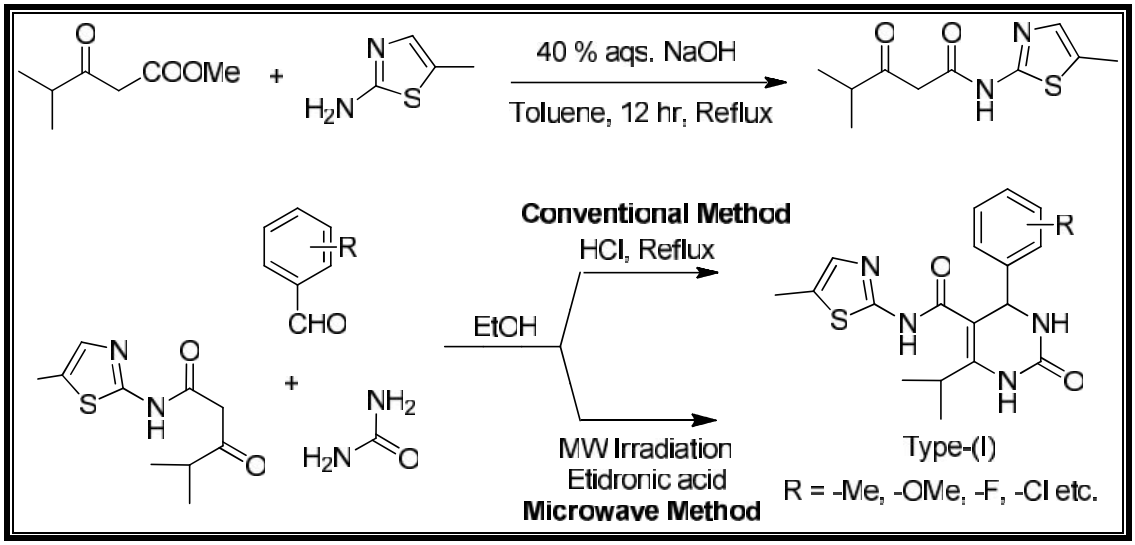
SYNTHESIS OF 4-ARYL-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE USING CONVENTIONAL METHOD & MICROWAVE ASSISTED METHOD AND BIOLOGICAL SCREENING.

Much interest has been focused around tetrahydropyrimidine derivatives because of their wide variety of pharmacological properties and industrial applications. In view of these reports, we have synthesized 4-aryl-6-isopropyl-*N*-(5-methyl-1,3-thiazol-2-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide of Type-(I) by the cyclocondensation reaction of 4-methyl-*N*-(5-methylthiazol-2-yl)-3-oxopentanamide, aromatic aldehyde and urea in ethanol.

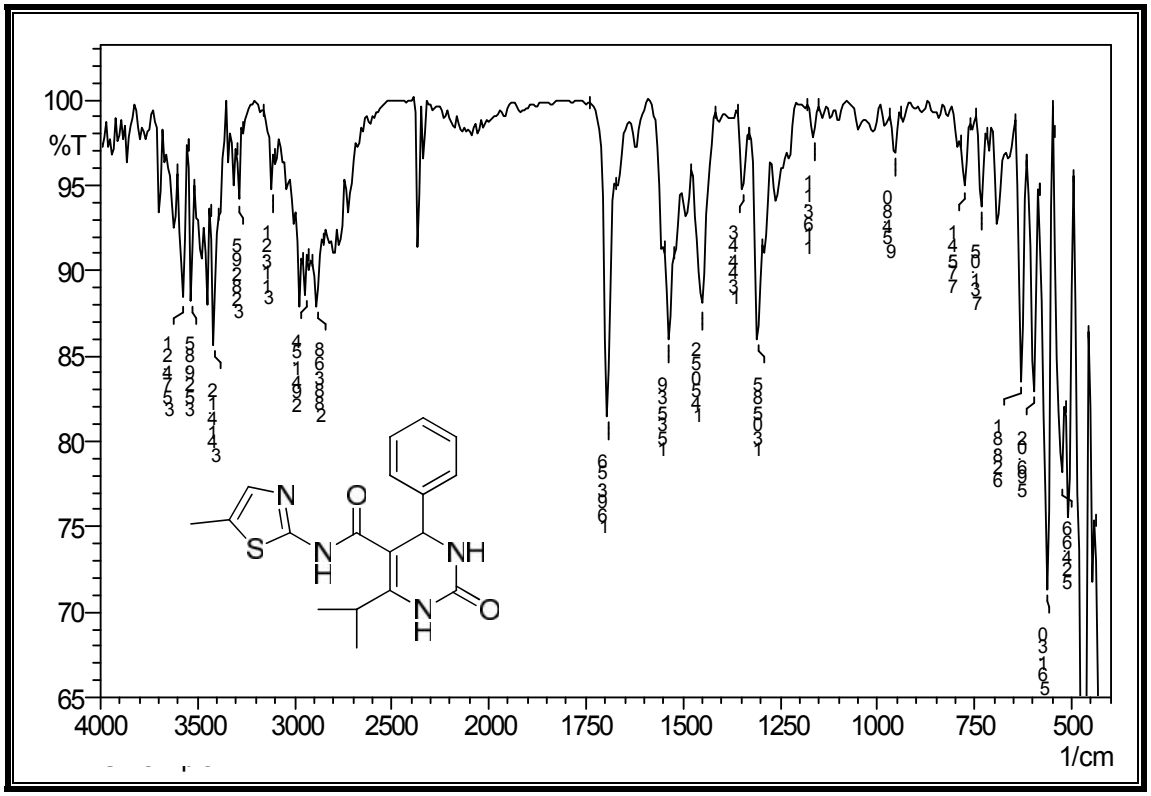
The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

REACTION SCHEME



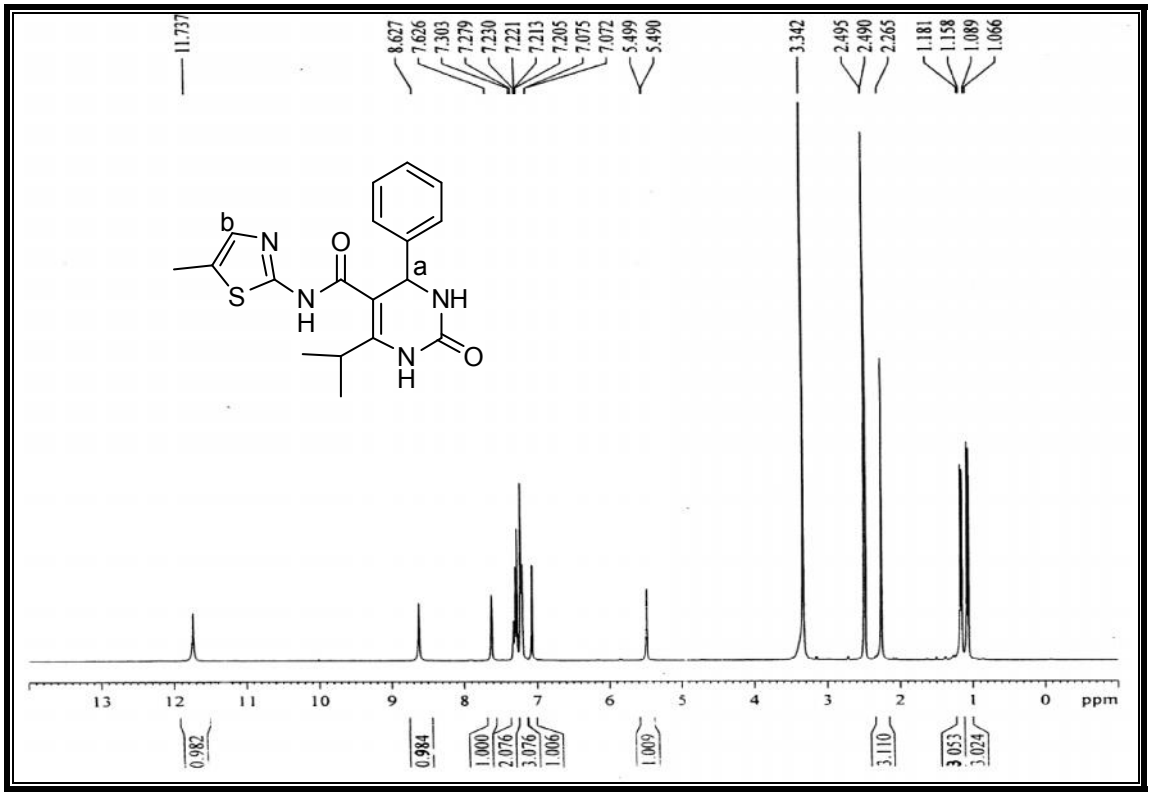
IR SPECTRUM OF 6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-4-PHENYL-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2941	2975-2920	112
	C-H str. (sym.)	2883	2880-2860	”
	C-H def. (asym.)	1450	1470-1435	”
	C-H def. (sym.)	1344	1395-1370	”
Aromatic	C-H str.	3113	3100-3000	”
	C=C	1535	1585-1480	”
	C-H i.p. def.	1163	1125-1090	”
	C-H o.o.p. def.	775	860-810	”
Carbonyl	C=O	1693	1700-1650	”
Amide	-NH str.	3414	3400-3200	”

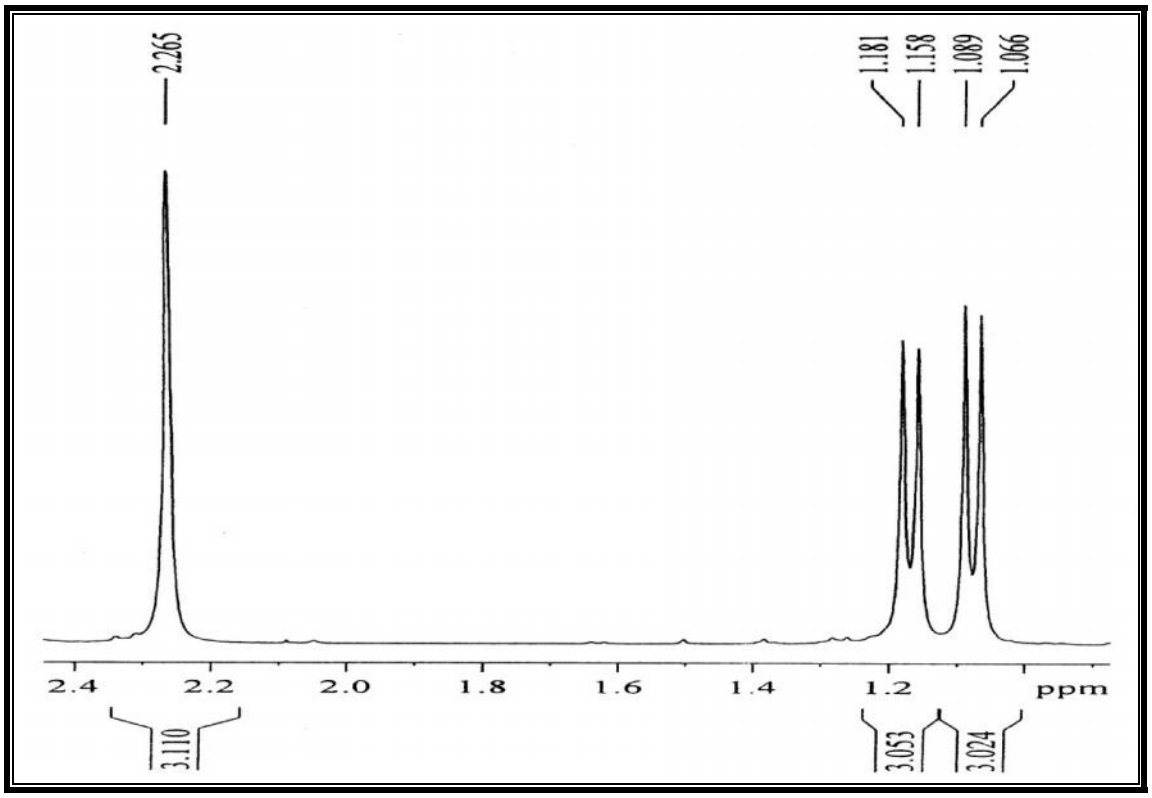
^{1}H-NMR SPECTRUM OF 6-ISOPROPYL- *N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-4-PHENYL-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

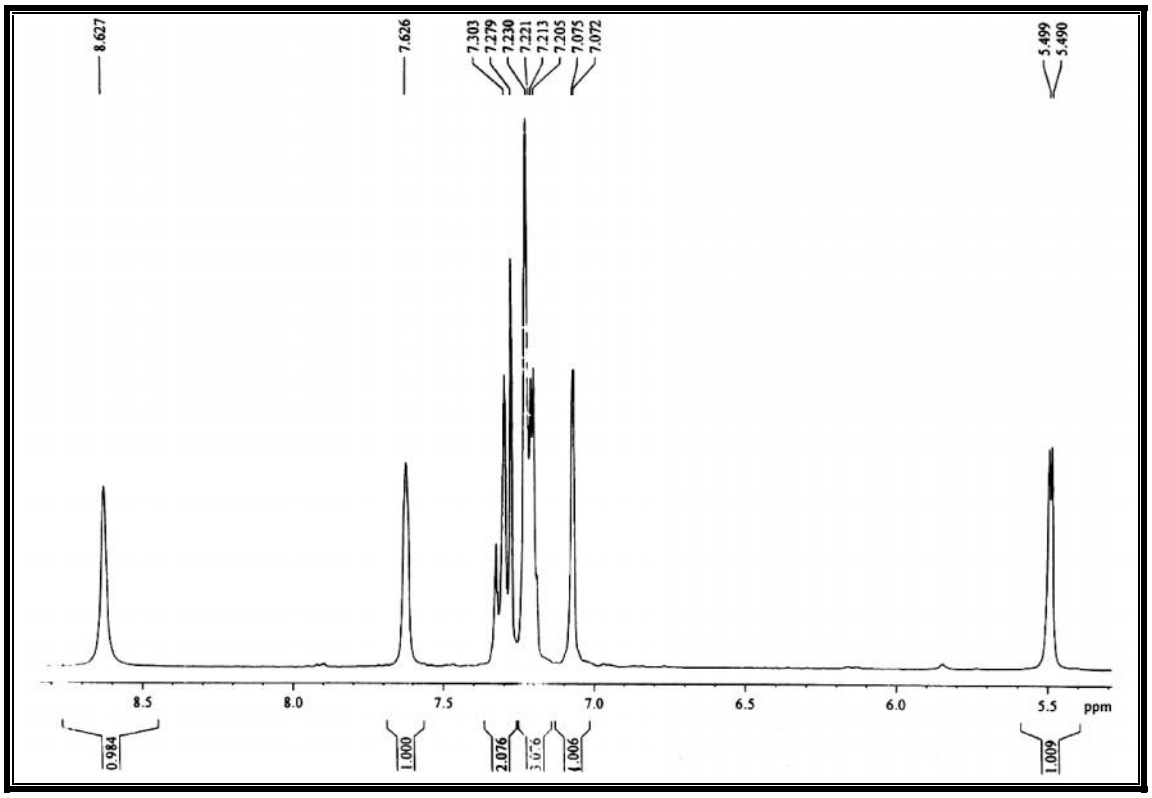


Internal Standard: TMS; Solvent: DMSO-D_6 Instrument: BRUKER Spectrometer (300MHz)

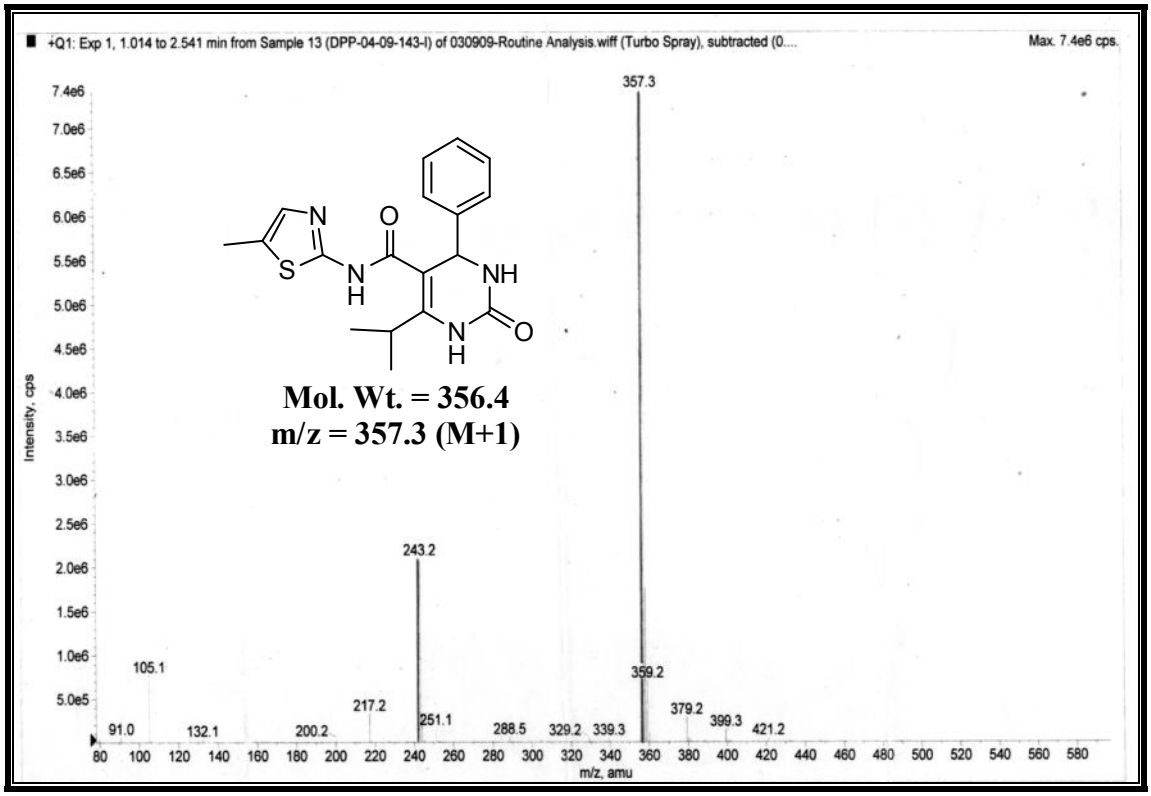
Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	1.06-1.08	3H	doublet	-CH(C<u>H</u>$_3$)$_2$	6.9
2	1.15-1.18	3H	doublet	-CH(C<u>H</u>$_3$)$_2$	6.9
3	2.26	3H	singlet	-CH_3	-
4	5.49	1H	doublet	H_a	2.7
5	7.07	1H	doublet	-CO-N<u>H</u>-	0.9
6	7.20-7.62	5H	multiplet	Ar-H	-
7	7.62	1H	singlet	H_b	-
8	8.62	1H	singlet	-CO-N<u>H</u>-	-
9	11.73	1H	singlet	-CO-N<u>H</u>-	-

EXPANDED ^{1}H-NMR SPECTRUM

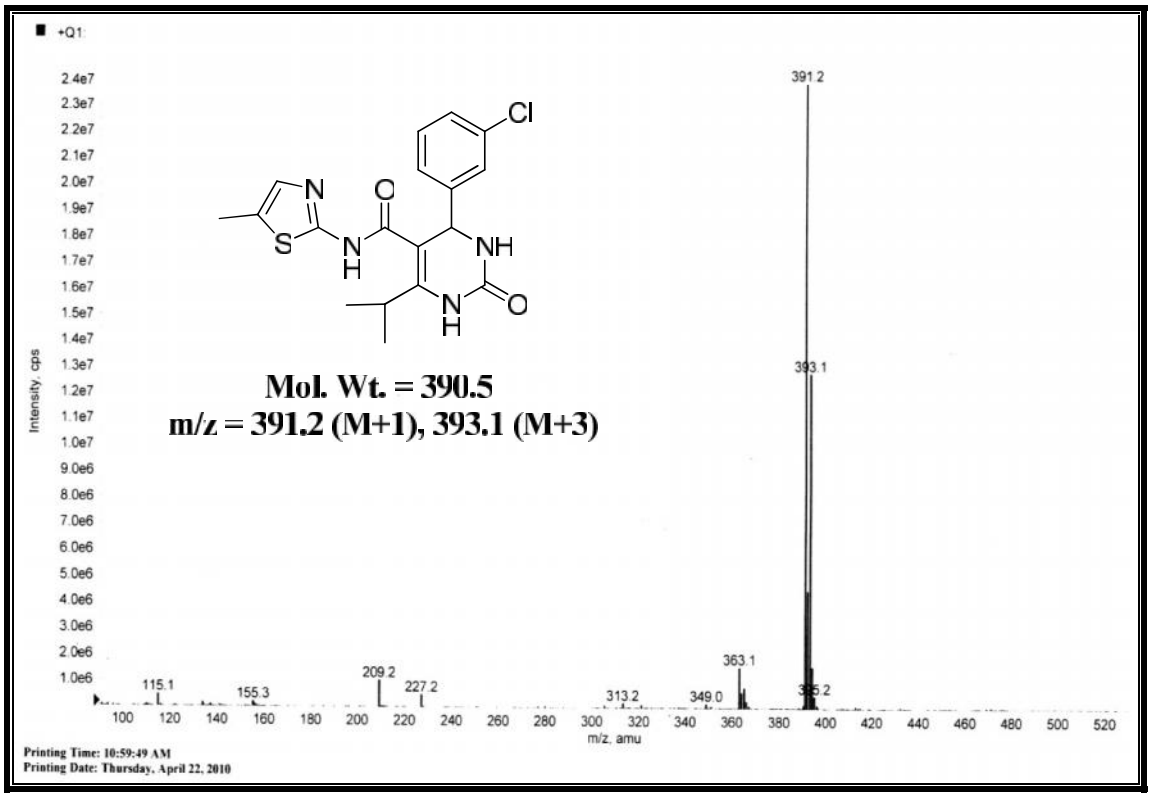




MASS SPECTRUM OF 6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-4-PHENYL-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE



MASS SPECTRUM OF 4-(3-CHLOROPHENYL)-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE



EXPERIMENTAL

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. Ethyl acetate:hexane (3:7) was used as a mobile phase.

[A] PREPARATION OF 4-METHYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-3-OXOPENTANAMIDE.

A suspension of methylisobutryl acetate (2.88 gm, 0.02 mol) and 5-methyl-1,3-thiazole-2-yl (1.14 gm, 0.01 mol) in toluene (20 ml) containing catalytic amount of NaOH solution (0.05 ml, 40 %) was reflux on oil bath for 12 hr. The progress of reaction was monitored by TLC, after completion of the reaction solvent was removed under reduced pressure to give residue, the residue was crystallized from mixture of hexane and ethyl acetate.Yield 51 %.

[B] PREPARATION OF 6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-4-PHENYL-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

(1) Conventional method

The warm mixture of 4-methyl-*N*-(5-methyl-1,3-thiazole-2-yl)-3-oxopentanamide (2.26 gm, 0.01 mole), benzaldehyde (1.06 gm, 0.01 mol), urea (0.9 gm, 0.015 mol) and ethanol (15 ml), in the presence of catalytic of concentrated HCl (2-3 drops) was stirred at reflux temperature for 12 hr. The progress of reaction was monitored by TLC, after completion of the reaction, the reaction mixture was allowed to stand at room temperature for several hours so precipitation was obtained. The product was filtered, washed with chilled methanol and isolated product was recrystallized from ethanol. Yield 62 %.

(2) Microwave assisted method

A well stirred mixture of 4-methyl-*N*-(5-methyl-1,3-thiazole-2-yl)-3-oxopentan amide (2.26 gm, 0.01 mole), benzaldehyde (1.06 gm, 0.01 mol), urea (0.9 gm, 0.015 mol) and ethanol (15 ml) in the presence of etidronic acid (100 mg/0.01 mol) was irradiated under microwave oven for 8 min at 300W. The progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was allowed to stand at room

temperature for several hours so precipitation was obtained. The product was filtered, washed with chilled methanol and isolated product was recrystallized from ethanol. Yield 89 %.

Similarly, other 6-isopropyl-*N*-(5-methyl-1,3-thiazol-2-yl)-4-aryl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide **(1a-j)** were prepared. The physical constants are recorded in **Table-1a,** page no. 39.

[C] BIOLOGICAL SCREENING OF 4-ARYL-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

➢ DETERMINATION OF MINIMAL INHIBITION CONCENTRATIONS BY BROTH DILUTION METHOD

All the synthesized compounds **(1a-j)** were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method[113-115] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96 and *Streptococcus pyogenes* MTCC 442, two Gram-negative bacteria *Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 1688 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323 taking gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all synthesized compounds **(1a-j)**, defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards[113].

Minimal Inhibition Concentration [MIC]

The main advantage of the **Broth Dilution Method** for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 ^{0}C overnight.

3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.
5. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

Methods used for primary and secondary screening

Each sample was diluted obtaining 2000 μg mL^{-1} concentration, as a stock solution. Inoculum size for test strain was adjusted to 10^8 cfu (colony forming unit) per milliliter by comparing the turbidity.

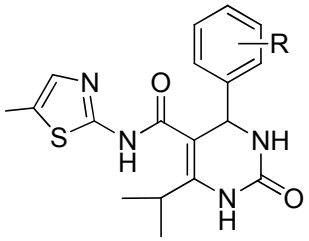
Primary screen: In primary screening 1000 μg mL^{-1}, 500 μg mL^{-1} and 250 μg mL^{-1} concentrations of the synthesized compounds were taken. The active samples found in this primary screening were further tested in a second set of dilution against all microorganisms.

Secondary screen: The samples found active in primary screening were similarly diluted to obtain 200 μg mL^{-1}, 100 μg mL^{-1}, 62.5 μg mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1} and 6.250 μg mL^{-1} concentrations.

Reading Result: The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain 10^8 organism/mL.

The results obtained from antimicrobial testing are recorded in **Table-1b,** page no. 40.

TABLE-1a: PHYSICAL CONSTANTS OF 4-ARYL-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE



Sr. No.	R	Molecular Formula/ Molecular Weight	MP °C	Classical method		Microwave Method		% Composition Calcd./Found		
				Yield %	Time (hr.)	Yield %	Time (min.)	C	H	N
1a	H	$C_{18}H_{20}N_4O_2S$ 356.44	219-221	62	12	89	8	60.65 60.34	5.66 5.59	15.72 15.55
1b	4-OMe	$C_{19}H_{22}N_4O_3S$ 386.47	229-331	54	11	76	6	59.05 58.76	5.74 5.65	14.50 14.38
1c	4-F	$C_{18}H_{19}FN_4O_2S$ 374.43	207-209	59	14	78	10	57.74 57.37	5.11 5.08	14.96 14.81
1d	3-Cl	$C_{18}H_{19}ClN_4O_2S$ 390.89	204-206	51	16	81	14	55.31 55.14	4.90 4.78	14.33 14.11
1e	2,3-di Cl	$C_{18}H_{18}Cl_2N_4O_2S$ 425.33	231-232	53	12	69	8	50.83 50.54	4.27 4.16	13.17 13.05
1f	4-$N(Me)_2$	$C_{20}H_{25}N_5O_2S$ 399.51	191-194	56	11	71	8	60.13 59.95	6.31 6.19	17.53 17.36
1g	2,5-di OMe	$C_{20}H_{24}N_4O_4S$ 416.49	245-247	47	14	67	12	56.68 56.31	5.81 5.74	13.45 13.29
1h	4-NO_2	$C_{18}H_{19}N_5O_4S$ 401.44	211-214	60	13	87	10	53.85 53.62	4.77 4.65	17.45 17.32
1i	3-NO_2	$C_{18}H_{19}N_5O_4S$ 401.44	223-225	45	12	83	8	53.85 53.62	4.77 4.62	17.45 17.26
1j	4-OH	$C_{18}H_{20}N_4O_3S$ 372.44	246-247	54	15	77	12	58.05 57.81	5.41 5.39	15.04 14.88

TABLE-1b: BIOLOGICAL SCREENING OF 4-ARYL-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	1a	250	250	250	250	1000	250	250
2	1b	500	250	**62.5**	250	**100**	**100**	250
3	1c	**200**	100	500	500	500	1000	1000
4	1d	250	500	100	200	250	500	1000
5	1e	500	250	500	500	200	500	500
6	1f	250	500	250	250	250	>1000	>1000
7	1g	250	500	200	100	250	500	500
8	1h	**200**	500	200	250	500	1000	1000
9	1i	250	500	250	250	250	1000	1000
10	1j	500	250	250	500	500	>1000	>1000

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted Pyrimidines **1c** (R= 4-F) & **1h** (R= 4-NO_2) against *S.aureus* and **1b** (R= 4-OMe) against *E-coli* possess excellent activity as compare to ampicillin. While **1c** (R= 4-F) against *S.pyogenus*, **1d** (R= 3-Cl) against *E-coli* and **1g** (R= 2,5-di OMe) against *P.aeruginosa,* possess good activity as compare with ampicillin. The remaining compounds possess moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted Pyrimidine **1b** (R= 4-OMe) show highly promising activity against *C.albicans* & *A.niger* compare with greseofulvin. While **1e** (R = 2,3-di Cl) against *C.albicans*, **1a** (R = -H) against *A.niger* and **1a** (R = -H) & **1b** (R= 4-OMe) against *A.clavatus,* possess good activity compare to standard drug. The remaining compounds exhibite moderate to poor activity.

SECTION-II

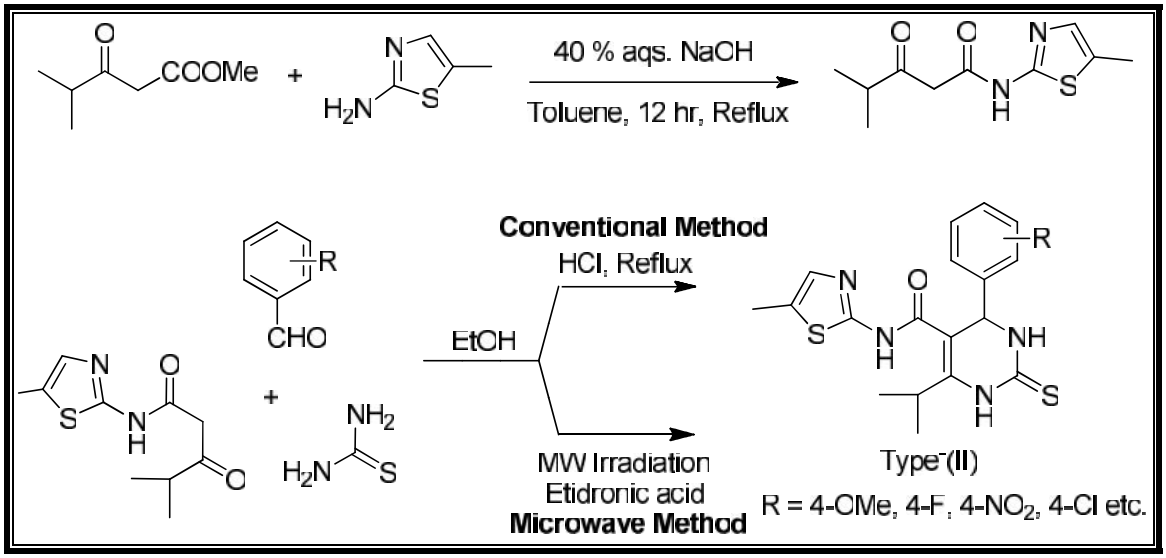
SYNTHESIS OF 4-ARYL-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE USING CONVENTIONAL METHOD & MICROWAVE ASSISTED METHOD AND BIOLOGICAL SCREENING.

Compounds containing pyrimidine moiety are widely distributed in nature. Many of these derivatives are reported to possess different biological activities. In view of these reports, we have synthesized 4-aryl-6-isopropyl-*N*-(5-methyl-1,3-thiazol-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide of Type-(II) by the cyclocondensation reaction of 4-methyl-*N*-(5-methylthiazol-2-yl)-3-oxopentanamide, aromatic aldehyde and thiourea in ethanol.

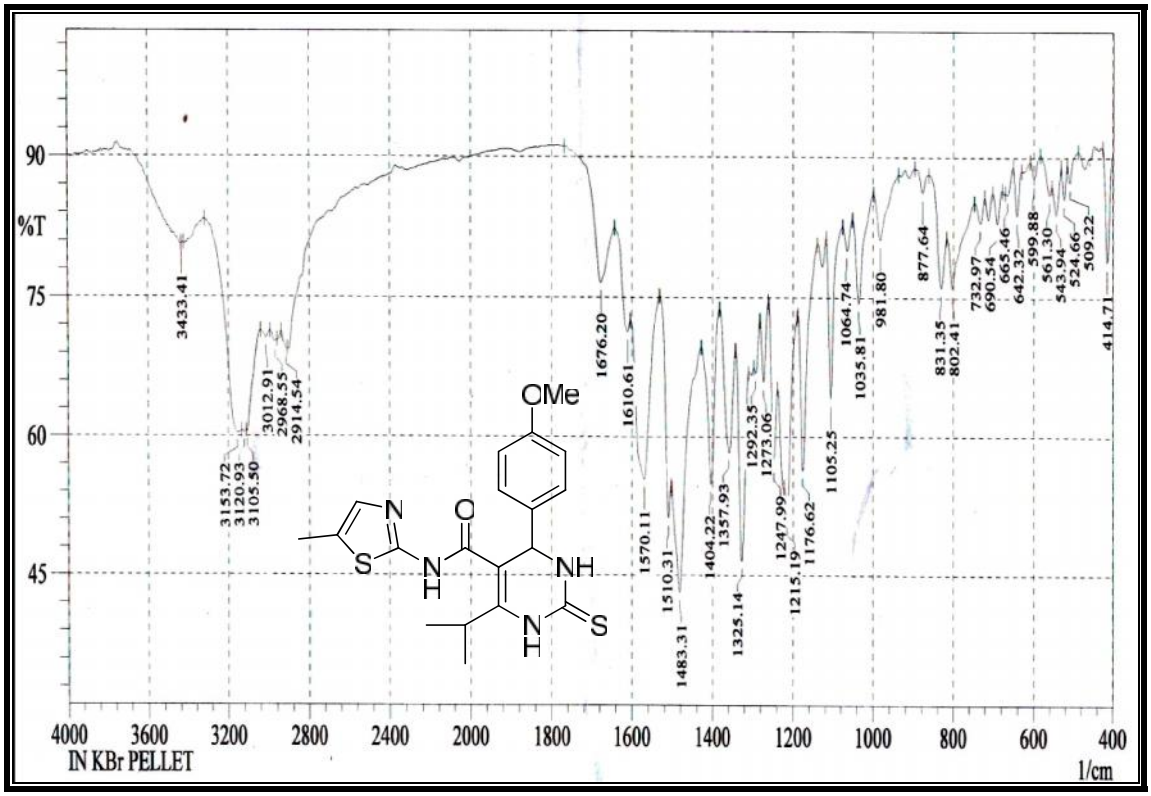
The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

REACTION SCHEME



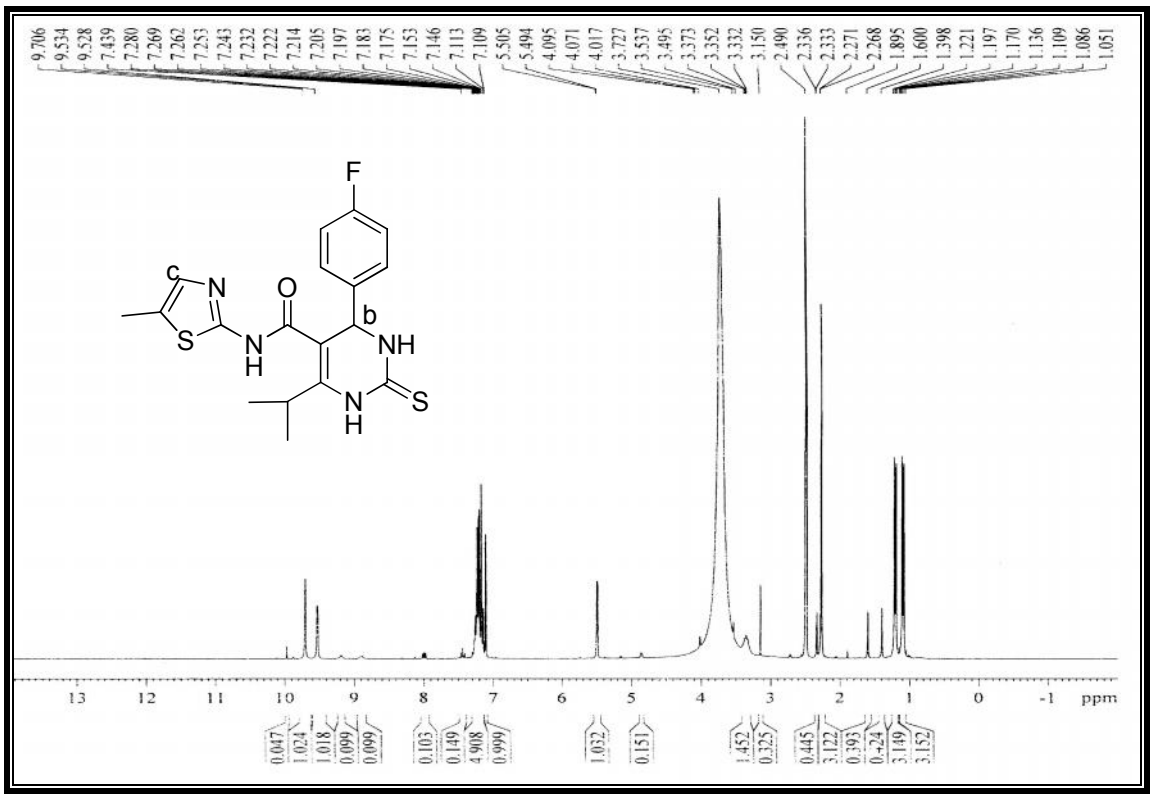
IR SPECTRUM OF 6-ISOPROPYL-4-(4-METHOXYPHENYL)-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDRO-PYRIMIDINE-5-CARBOXAMIDE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2968	2975-2920	112
	C-H str. (sym.)	2914	2880-2860	”
	C-H def. (asym.)	1483	1470-1435	”
	C-H def. (sym.)	1357	1395-1370	”
Aromatic	C-H str.	3105	3100-3000	”
	C=C	1570	1585-1480	”
	C-H i.p. def.	1105	1125-1090	”
	C-H o.o.p. def.	831	860-810	”
Carbonyl	C=O	1676	1700-1650	”
Amide	-NH str.	3433	3400-3200	”

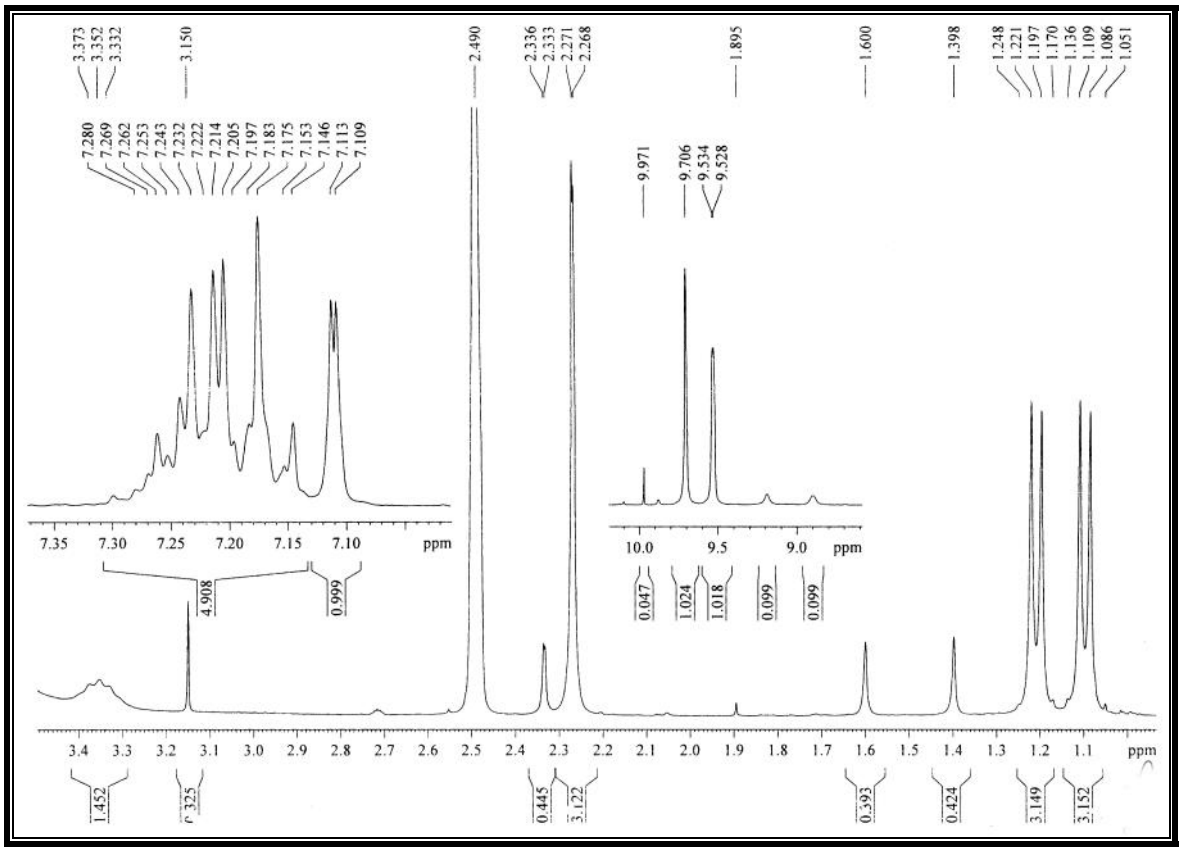
^{1}H-NMR SPECTRUM OF 4-(4-FLUOROPHENYL)-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE



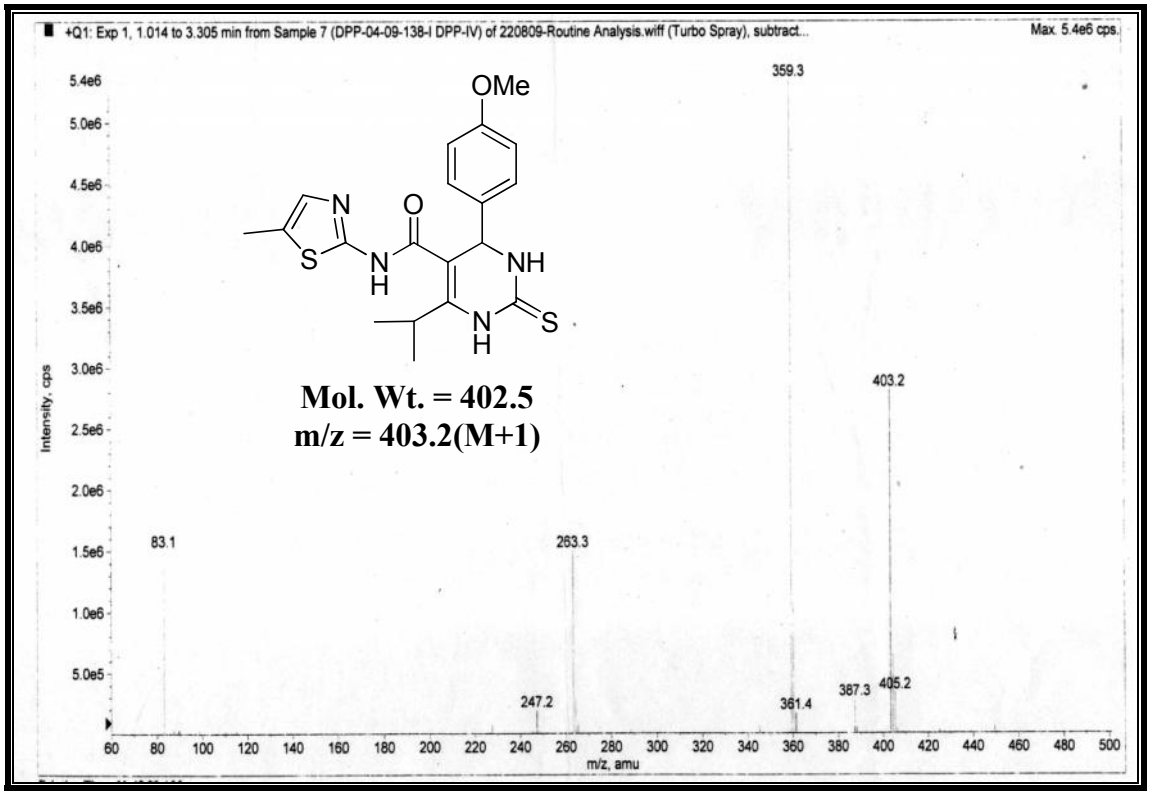
Internal Standard: TMS; Solvent: DMSO-D_6 Instrument: BRUKER Spectrometer (300MHz)

Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	1.08-1.10	3H	doublet	-CH(C<u>H</u>$_3$)$_2$	-
2	1.19-1.22	3H	doublet	-CH(C<u>H</u>$_3$)$_2$	-
3	2.26	3H	singlet	-CH_3	-
5	3.35	1H	multiplet	-C<u>H</u>(CH$_3$)$_2$	-
6	5.49-5.50	1H	doublet	Chiral-H_b	3.3
7	7.10-7.11	1H	doublet	-CO-NH-	1.2
8	7.14-7.28	5H	multiplet	Ar-H+H_c	-
9	9.52+9.53	1H	doublet	-CO-N<u>H</u>-	1.8
10	9.70	1H	singlet	-CO-N<u>H</u>-	-

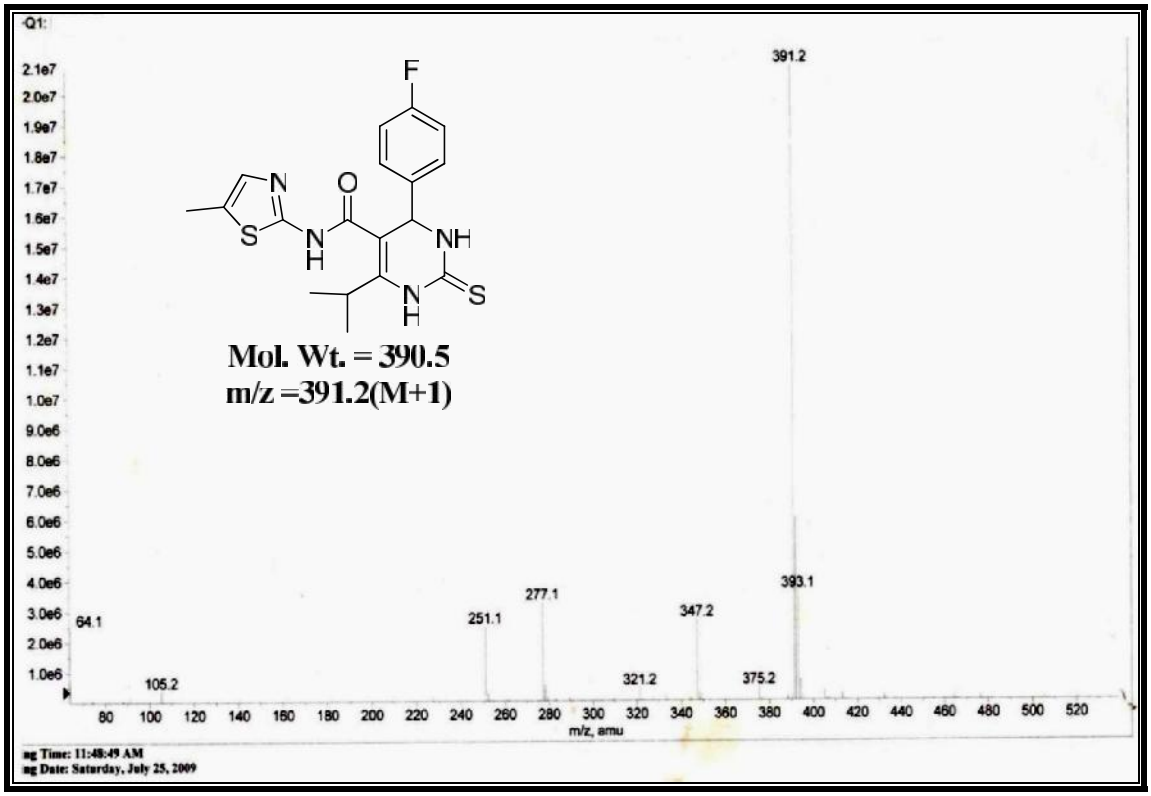
EXPANDED ^{1}H-NMR SPECTRUM



MASS SPECTRUM OF 6-ISOPROPYL-4-(4-METHOXYPHENYL)--*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE



MASS SPECTRUM OF 4-(4-FLUOROPHENYL)-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE



EXPERIMENTAL

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. Ethyl acetate:hexane (3:7) was used as a mobile phase.

[A] PREPARATION OF 4-METHYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-3-OXOPENTANAMIDE.

See Chapter-1, Section-I, Experimental [A], Page no. 36.

[B] PREPARATION OF 6-ISOPROPYL-4-(4-METHOXYPHENYL)-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

(1) Conventional method

The warm mixture of 4-methyl-*N*-(5-methyl-1,3-thiazole-2-yl)-3-oxopentanamide (2.26 gm, 0.01 mole), 4-methoxy benzaldehyde (1.36 gm, 0.01 mol) and thiourea (1.14 gm, 0.015 mol) in ethanol (15 ml), containing 3-4 drops of concentrated HCl was stirred under reflux for 13 hr. The progress of reaction was monitored by TLC, after completion of the reaction, the reaction mixture was allowed to stand at room temperature for several hours so precipitation was obtained. The product was filtered, washed with chilled methanol and isolated product was recrystallized from ethanol. Yield 52 %.

(2) Microwave assisted method

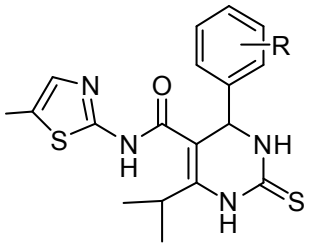
A well stirred mixture of 4-methyl-*N*-(5-methyl-1,3-thiazole-2-yl)-3-oxopentanamide (2.26 gm, 0.01 mole), 4-methoxy benzaldehyde (1.36 gm, 0.01 mol) and thiourea (1.14 gm, 0.015 mol) in ethanol (15 ml) in the presence of etidronic acid (100 mg/0.01 mol) was irradiated under microwave oven for 10 min at 300W. The progress of reaction was monitored by TLC, after completion of reaction, the reaction mixture was allowed to stand at room temperature for several hours so precipitation was obtained. The product was filtered, washed with chilled methanol and isolated product was recrystallized from ethanol. Yield 78 %.

Similarly, other 4-aryl-6-isopropyl-*N*-(5-methyl-1,3-thiazol-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide **(2a-j)** were prepared. The physical constants are recorded in **Table-2a,** page no.49.

[C] BIOLOGICAL SCREENING OF 4-ARYL-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4- TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE.

Antimicrobial testing was carried out as described in Chapter-1**,** Section-I, Experimental [C], page no. 37. The results obtained from antimicrobial testing are recorded in **Table-2b,** page no. 50.

TABLE-2a: PHYSICAL CONSTANTS OF 4-ARYL-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDRO-PYRIMIDINE-5-CARBOXAMIDE



Sr. No.	R	M.F. / M.W.	MP °C	Classical method		Microwave Method		% Composition Calcd./Found		
				Yield %	Time (hr.)	Yield %	Time (min.)	C	H	N
2a	4-OMe	$C_{19}H_{22}N_4O_2S_2$ 402.53	239-242	60	13	81	10	56.59 56.33	5.51 5.34	13.92 13.67
2b	H	$C_{18}H_{20}N_4OS_2$ 372.51	227-229	56	15	85	8	58.04 57.81	5.41 5.31	15.04 14.88
2c	4-F	$C_{18}H_{19}FN_4OS_2$ 390.50	217-218	55	14	76	7	55.36 55.09	4.90 4.86	14.35 14.23
2d	3-Cl	$C_{18}H_{19}ClN_4OS_2$ 406.95	198-201	41	17	77	9	53.12 53.08	4.71 4.59	13.77 13.51
2e	2,3-di Cl	$C_{18}H_{18}Cl_2N_4OS$ 441.40	224-226	49	16	63	16	48.98 48.71	4.11 4.04	12.69 12.47
2f	4-$N(Me)_2$	$C_{20}H_{25}N_5OS_2$ 415.58	208-210	55	12	68	8	57.80 57.55	6.06 5.94	16.85 16.69
2g	2,5-di OMe	$C_{20}H_{24}N_4O_3S_2$ 432.56	229-231	43	20	64	18	55.53 55.26	5.59 5.47	12.95 12.81
2h	4-NO_2	$C_{18}H_{19}N_5O_3S_2$ 417.51	222-225	54	13	82	14	51.78 51.42	4.59 4.41	16.77 16.52
2i	3-NO_2	$C_{18}H_{19}N_5O_3S_2$ 417.51	231-234	48	15	79	17	51.78 51.58	4.59 4.45	16.77 16.60
2j	4-OH	$C_{18}H_{20}N_4O_2S_2$ 388.51	252-253	51	19	74	13	55.65 55.28	5.19 5.07	14.42 14.16

TABLE-2b: BIOLOGICAL SCREENING OF 4-ARYL-6-ISOPROPYL-*N*-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDRO-PYRIMIDINE-5-CARBOXAMIDE

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	2a	**200**	200	200	250	500	250	250
2	2b	500	500	200	250	500	500	1000
3	2c	250	250	100	100	**250**	500	500
4	2d	250	250	200	**62.5**	1000	500	500
5	2e	500	100	**62.5**	250	500	1000	1000
6	2f	500	500	250	500	500	1000	>1000
7	2g	**200**	200	500	500	500	1000	1000
8	2h	500	500	250	250	1000	>1000	>1000
9	2i	500	500	250	250	500	1000	1000
10	2j	500	500	200	250	500	>1000	>1000

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted Pyrimidines **2a** (R= 4-OMe) & **2g** (R= 2,5-di OMe) against *S.aureus*, **2e** (R= 2,3-di Cl) against *E-coli* and **2d** (R= 3-Cl) against *P.aeruginosa* possess very good activity compare to ampicillin. While **2c** (R= 4-F) & **2d** (R= 3-Cl) against *S.aureus,* **2e** (R= 2,3-di Cl) against *S.pyogenus* and **2c** (R= 4-F) against *E-coli* & *P.aeruginosa,* possess moderate activity as compare with ampicillin. The remaining compounds possess moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted Pyrimidine **2c** (R= 4-F) exhibite promissing activity against *C.albicans* while **2a** (R= 4-OMe) exhibite moderate activity against *A.niger* & *A.clavatus* compare to Greseofulvin. The remaining compounds exhibite moderate to poor activity.

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Chapter-2, Part-1

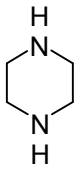
STUDIES ON PIPERAZINE DERIVATIVES

INTRODUCTION

Piperazine was used early in the 20th century for the treatment of gout. Giround discovered the anthelmintic activity of piperazine, synthesized by Cloez in 1853, fortuitously in 1942; the same effect was observed by Biosmare in 1948 and conformed by Bayared in 1949. Structural modification of this molecule in the search for effective filaricidal resulted in diethyl carbamazine synthesized by Kishner et al in 1946 and studied pharmacologically by Hewitt et al in 1947. Clinical studies have shown that the drug is highly effective against both ascaris lumbricoides and enterobius (oxyuris) vermicularis.

CHEMISTRY

Piperazine is a six member saturated cyclic secondary diamine with the nitrogen in the 1, 4 - position. It can also be called as Hexahydropirazine, 1,4-Diazacyclohexane, 1,4-Diethylenediamine, Diethyleneimine or Antiren, its molecular formula is $C_4H_{10}N_2$, its molecular weight is 86.14 and structural formula shown as under (1).



(1)

PHYSICAL PROPERTIES

Piperazine is available in the form of hygroscopic colourless crystals or white flakes, with pungent odour, it is soluble in water and ethanol, but insoluble in ether. An aqueous solution reacts strongly alkaline, the pka being 9.8, M.P. 106 °C, B.P. 146 °C and density is 1.1 gm/ml. It is commonly available industrially as the hexahydrate (M.F.-$C_4H_{10}N_2$. $6H_2O$, M.P. 44°C, B.P. 125-130°C) which contains about 44 % of base and in addition, Two common salts in the form of which piperazine is usually prepared for pharmaceutical or veterinary purposes are the citrate (M.F.-$3C_4H_{10}N_2.2C_6H_8O_7$, M.P. 182-187 °C) and the adipate (M.F.-$C_4H_{10}N_2.C_6H_{10}O_4$, M.P. 256-257 °C).

PHARMACOLOGICAL EFFECT

Piperazine was first introduced as an anthelmintic. A large number of piperazine derivatives have anthelmintic action. Their mode of action is generally by paralysing parasites, which allows the host body to easily remove or expel the invading organism.

This action is mediated by its agonist effects upon the inhibitory GABA (γ-aminobutyric acid) receptor.

Orally administrated piperazine is almost devoid of pharmacological activity. Intravenous administration results in a transient fall in blood pressure. Lethal doses cause convulsions and respiratory depression.

In addition to anthelmintic activity, piperazine derivatives possess various pharmacological activities such as antihistamines, antipsychotics, antianginals, anti-depressants, antihypertensives, anesthetics, analgesics, anticonvulsants, antispasmodics and urologicals.

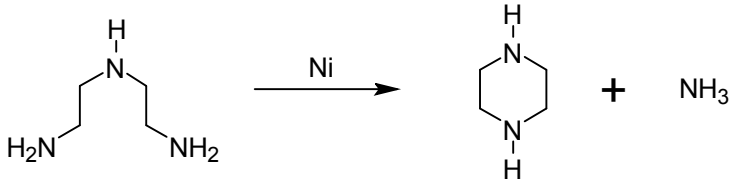
Piperazine and its derivatives are also used in the manufacturing of rubbers, anti-oxidants, corrosion inhibitors, additives, cosmetics, dyeing agents, plastics, resins, pesticides, polymers, synthetic fibers and analytical reagents.

SYNTHETIC ASPECT

piperazine itself can be synthesized by reacting alcoholic ammonia with 1,2-dichloroethane, by the action of sodium and ethylene glycol on ethylene diamine hydrochloride, or by reduction of pyrazine with sodium in ethanol. However Industrially piperazine is synthesized by different method, some of them are described as below.

(A) Preparation of piperazine at atmospheric pressure;

N-(2-aminoethyl)ethane-1,2-diamine was heated with Raney nickel under various experimental conditions. In all cases ammonia was evolved and piperazine formed according to the reaction. A temperature at about 150 ^{0}C or somewhat higher was found to be most suitable for the reaction.

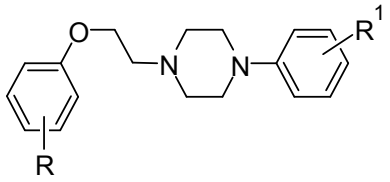


(B) Autoclave method for the preparation of piperazine;

The autoclave was charged with 150 gm of 2-((2-aminoethyl)amino)ethanol in 200 ml of dioxane 15 gm of catalyst was added, and reaction was carried out for 3 hours at a temperature in the range of 200-300 ^{0}C. The reaction mixture was filtered to remove catalyst and distilled; dioxane-water azeotrope distilled at 87 ^{0}C, then dioxane at 100-103 ^{0}C, and finally piperazine at 140-150 ^{0}C. Raney nickel appeared the catalyst at choice; copper chromium oxide, activated alumina, silica gel, cupric oxide and iron, were intermediate in effectiveness[1-9].

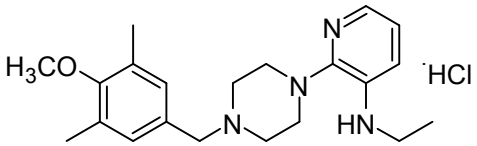
THERAPEUTIC IMPORTANCE

Rastogi et al[10] prepared N-(2-substituted-ethyl)-N$^{|}$-aryl piperazine (2) as potential antihookworm agents.



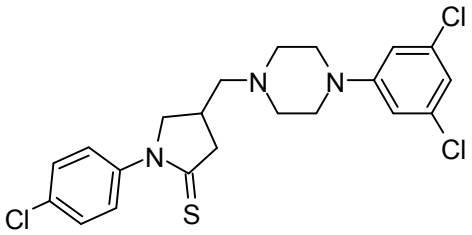
(2)

A variety of analogues of 1-[4-methoxy-3,5-dimethyl benzyl]-4-[3-(ethyl amino)-2-pyridyl]piperazine hydrochloride (3) was synthesized & evaluated for their anti HIV activity by N. Serradji et al[11].



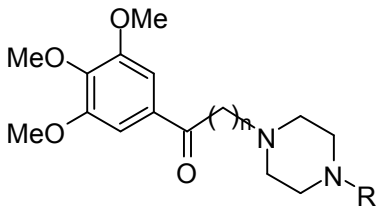
(3)

H. J. Shaue et al[12] synthesized a series of triazolopyridine derivatives, in order to explore effects of modifications of the alkyl piperazine moiety of triazolone on binding affinity for $5HT_{2A}$ & α_1 receptor. Hydrochlorides of compound (4) have been tested for their antispasmodic and antihistaminic properties and found to possess good activities comparable to standard compounds now used in medicinal practice[13].



(4)

R. B. Petigara et al[14] synthesized various N^1-N^4-substituted piperazine derivatives (5) and reported their CNS depressant activity.

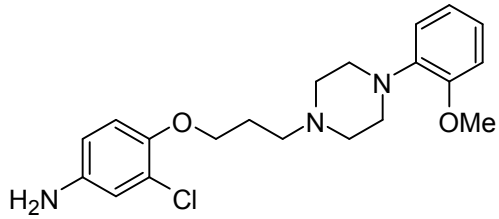


Where n= 0,1,2,3

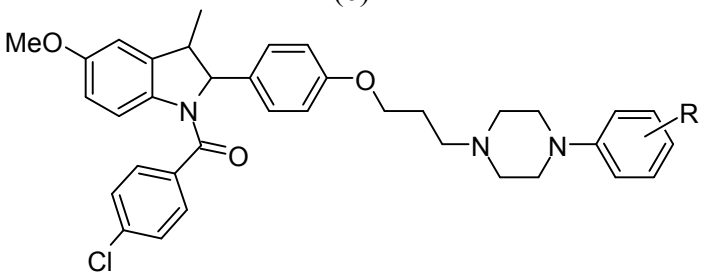
R=methyl, cyclohexyl, phenyl or benzyl

(5)

S. K. Saxena et al[15] synthesized piperazine derivatives (6) and B. M. Khadilkar et al[16] synthesised (7) showed best CNS depressant, anti-inflammatory and diuretic ativities**.** R. C. Tripathi et al[17] have been synthesized piperazine derivatives and evaluated its hypotensive and CNS depressant activities.

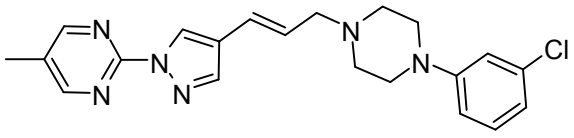


(6)

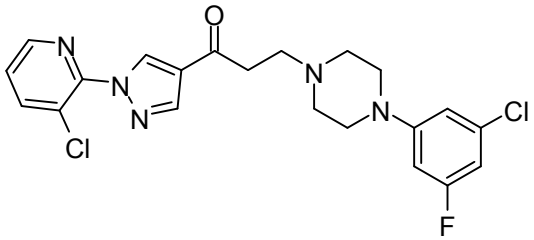


(7)

A. Ejima and S. Ohsuki[18] suggested that pyrazole derivatives (8) and (9) containing substituted piperazine nucleus possess good antitumor activity.

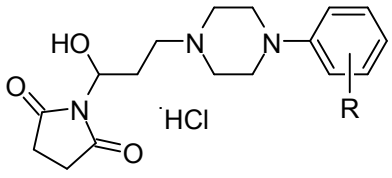


(8)



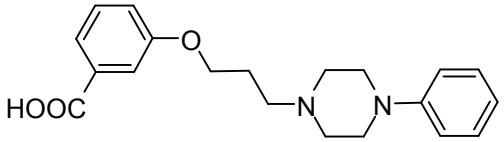
(9)

The effect of piperazine derivative (10) on blood pressure of cat was studied by B. M. Khadilkar & S. D. Samant [19] for intracerebroventricular administration at the dose of 50-µg/kg weights. The fall in B.P. was so rapid that cat died within 15 minutes.



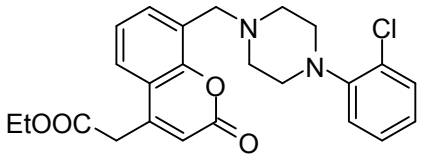
(10)

S. K. Agrawal et al[20] prepared some novel piperazine derivatives (11), some of these compounds show potent CNS depressant, hypotensive & α-adrenoceptor blocking activities.



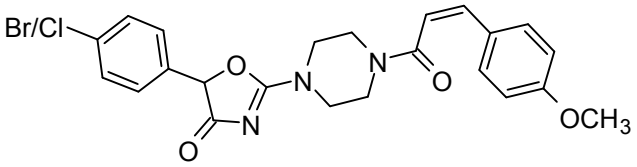
(11)

Korgaonkar et al[21] have prepared ethyl-8-[4-(3-chloro)-1-piperazinyl methyl]-7-hydroxycoumarin-4-acetate (12) as most effective antiinflammatory agents. Analgesic and antiinflammatory activities of piperazines reported by several researcher.[22-27]



(12)

Preparation of (13) and terir activity against *plasmodium berghei* were described by Herrin and his co-workers.[28] Some nitrogen containing heterocycles found to posses high antimalarial activity, which was described by F. S. Mikhalitsym et al.[29]

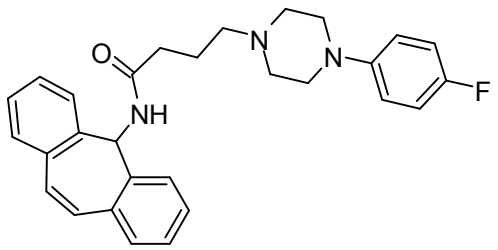


(13)

L. C. Meurer and his coworkers[30] synthesized some alkyl & halo substituted piperazine derivatives and studied their hypoglycemic activity. In these derivatives some compounds showed potent hypoglycemic activity. Varieties of hypoglycemic agents containing piperazine moiety were also assessed by several investigators.[31,32]

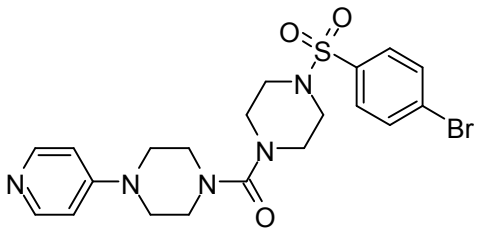
Some piperazine derivatives are showed a useful activities like as brain protectant[33], diagnosis of schizophrenia[34], neurokinine[35,36], serration antagonist receptors[37-39], heart muscle receptor[40], and cholesterol reducing agents[41], HIV-I inhibitors[42,43], dopamine transporter[44-46], sorbitol dehydrogenase inhibitor[47-49], spermicidal agent[50]. Besides the anathematic application of piperazine[51-53], anesthetic[54], antiparkinson[55], anticonvulsant[56-58], antidepressant agents[59], antipshycotic[60-61], antiallergic[62-64], antiulcer[65], insecticide[66], antiparasitics[67], normolipemic hypoglycemic & hypotriglyceridemic activity.[68]

M. Kurokawa et. al.[69] reported selective activity of 5-((4-(4-(Fluorophenyl)-1-piperazinyl)-butyryl)amino)-5*H*-dibenzo[a,d]cyclohepene) (14) for cardiac tissue over vascular tissue, thereby conferring antianginal activity without an effect on blood pressure.



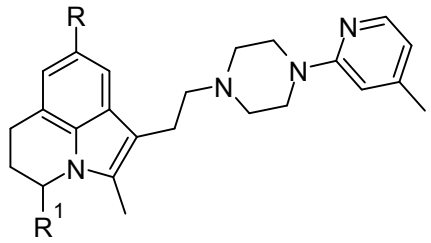
(14)

G. R. Brown[70] synthesized 1-(Arylsulfonyl)-4-{[1-(4-pyridyl)piperazine-1-yl] carbonyl}piperazines and analogs (15) and reported as oxido-sqelene cyclase inhibitors. 2-Piperazine-1-acetic acid derivatives are used as platelet aggregation inhibitors and antithrombotics[71], piperazinyl ethyl indazoles derivatived as calmodulin inhibitors[72] and piperazine carboxylic ester derivatives as tryptase inhibitors.[73]



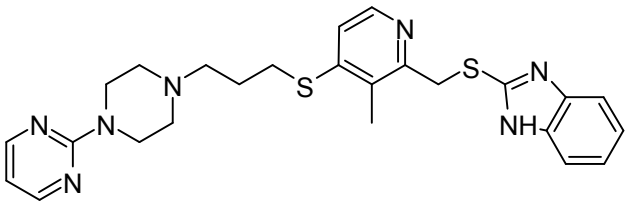
(15)

S. Paris et. al.[74] synthesized pyrrolo[3,2,1-i,j]quinoline (16) and documented as potential therapeutic application in asthma.



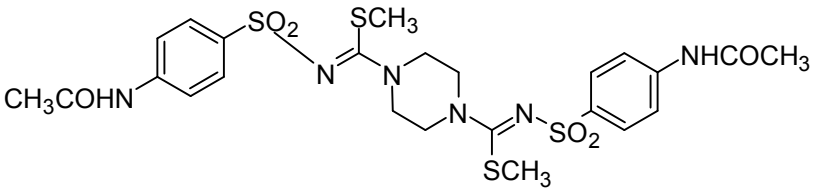
(16)

2-(((3-methyl-4-((3-(4-(pyrimidin-2-yl)piperazin-1-yl)propyl)thio)pyridin-2-yl)methyl)thio)-1H-benzo[d]imidazole (17) was recommended for the control of *helicobacter pybr*i bacteria by G. Hanauer and his co-workers.[75]

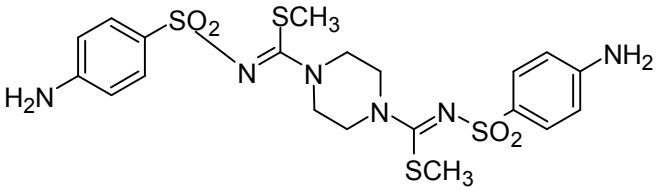


(17)

S. Seshadri et. al.[76] prepared dimeric products (18) and (19) of dithioacetals with piperazine showed moderate to significant activity. These compounds also show better activity against gram negative bacteria as compared to gram positive bacteria.

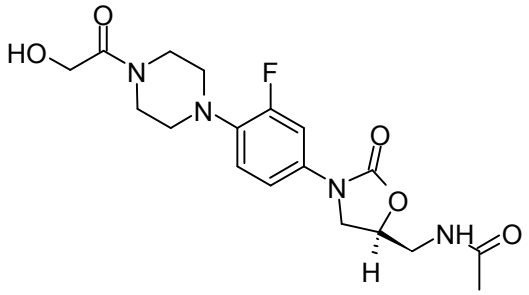


(18)



(19)

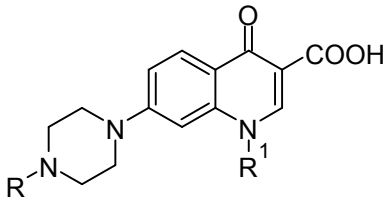
S. J. Brickner et. al.[77] prepared potent synthetic oxazolidin-2-one derivative (20) which is currently in clinical development for the gram positive bacterial infections caused by strains of S*taphylococci*, S*treptococci* & *Enterococci*. The *in vitro* and *in vivo* activities of above compound, against representative strains are similar to these of vancomycin. These compounds demonstrate potent *in vitro* activity against *mycobacterium tuberculosis*.



(20)

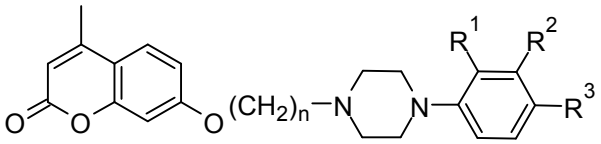
Some quinazolinone derivatives containing piperazines (21) found to posses strong antibacterial activity.[78-84] Substituted piperazinyl-phenyl-oxazolidione derivatives used as antibacterial agents, which showed MIC of 0.5 µg/ml against *S. auresas* by G. D.

Cung et. al.[85]



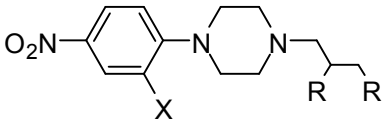
(21)

7-((4-Substitutedphenylpierazine-1-yl)-alkoxyl)-4-methylchromene-2-ones (22) as potential antipsychotic agents, synthesized and evaluated by S. H. Bhosale and his co-workers.[86]



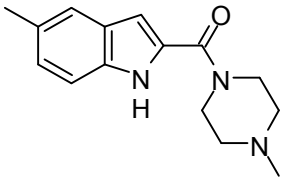
(22)

N^1-(2-Substituted-4-nitrophenyl)-N^4-(2,3-disubstitutedpropyl)-piperazines (23) as useful antifungal agent prepared and characterized by G. L. Talesara et. al.[87]



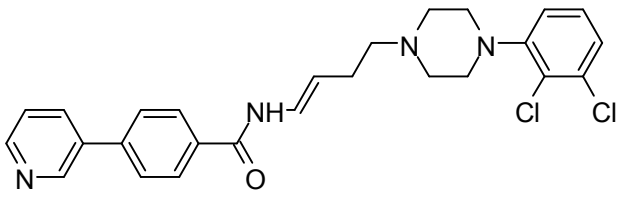
(23)

Preparation and biological evaluation of (5-methyl-1*H*-indol-2-yl)-4-methyl-piperazine-1-yl)-methanone (24) as potent human histamine H_4 antagonists by J. D. Venable and his co-workers.[88]



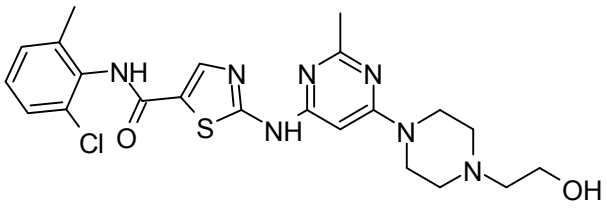
(24)

N-(4-(4-(2,3-Dichlorophenyl)-piperazine-1-yl)trans-but-2-enyl)-4-(3-pyridyl)-2-yl-benzamide (25) as selective probes with high affinity for the dopamine D3 receptor has been prepared by P. Grundt et. al.[89]



(25)

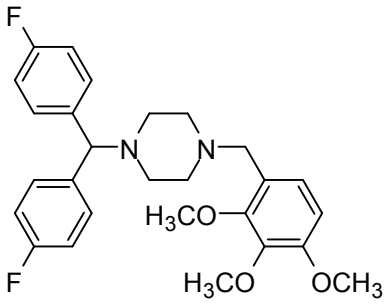
N-(2-chloro-6-methylphenyl)-2-((6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methyl pyrimidin-4-yl)amino)thiazole-5-carboxamide (26), a dual Src/Abl kinase inhibitors with potent antitumor activity in preclinical assays has been synthesized and discovered by L. J. Lombardo et al.[90]



(26)

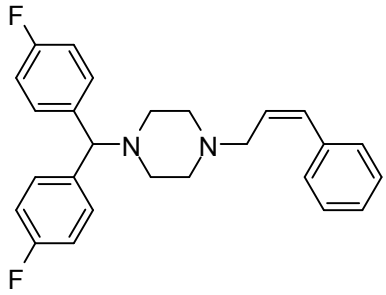
Several investigators synthesized varieties of new compounds with piperazine moiety and found their antimicrobial activities such as antifungal and antibacterial.[91-101]

Lomerizine(1-(2''',3''',4'''-trimethoxybenzyl)-4-[(4',4''-diflurodiphenyl)-Methyl)-piperazine) (27) is a calcium channel blocker with antimigraine properties.[102-106]



(27)

Flunarizine (brand name sibelium) {1-cinnamyl-4-[(4',4''-diflurodiphenyl)-methyl]-piperazine}(28) was discovered at Janssen Pharmaceutical in 1967. Flunarizine is a drug classified as a calcium channel blocker. Flunarizine is a selective calcium entry blocker with calmodulin binding properties and histamine H1 blocking activity by W. K. Amery.[107] It is effective in the prophylaxis of migraine, occlusive peripheral vascular disease, vertigo of central and peripheral origin, and as an adjuvant in the therapy of epilepsy. It may help to reduce the severity and duration of attacks of paralysis associated with the more serious form of alternating hemiplegia. Potent visodilater improve cerebral blood flow reduce duration and incidences of migraine attack by J. M. Van Nueten.[108]



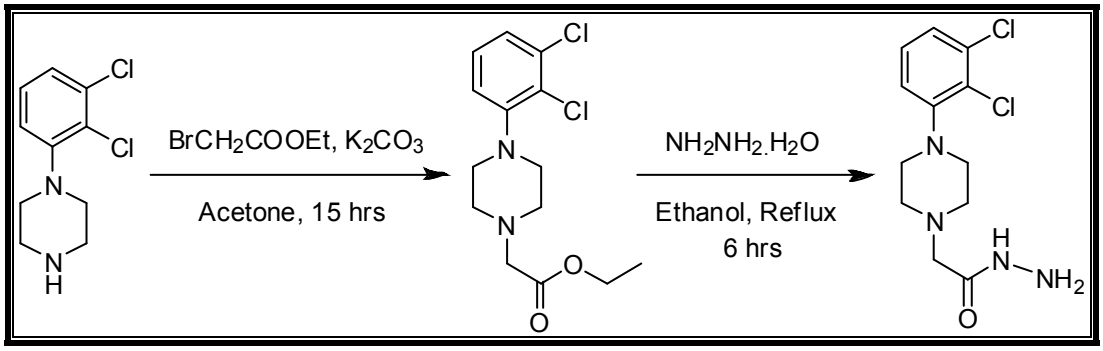
(28)

Thus the important role displayed by piperazine and its derivatives for various therapeutic and biological activities prompted us to synthesize some Schiff base, Thiazolidinone, Oxadiazole and Triazole derivatives bearing piperazine moiety in order to achive compounds having better biological activities.

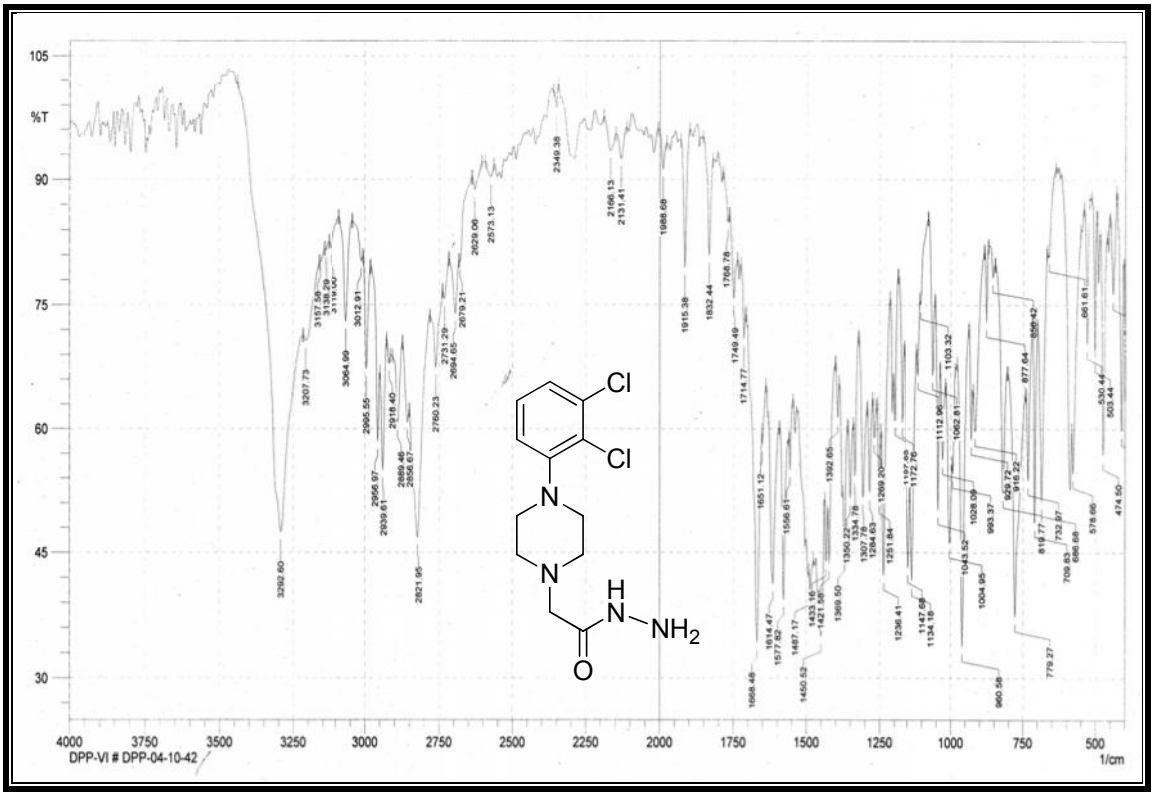
SECTION-I

SYNTHESIS AND CHARACTERIZATION OF 2-(4-(2,3-DICHLOROPHENYL) PIPERAZIN-1-YL)ACETOHYDRAZIDE

REACTION SCHEME



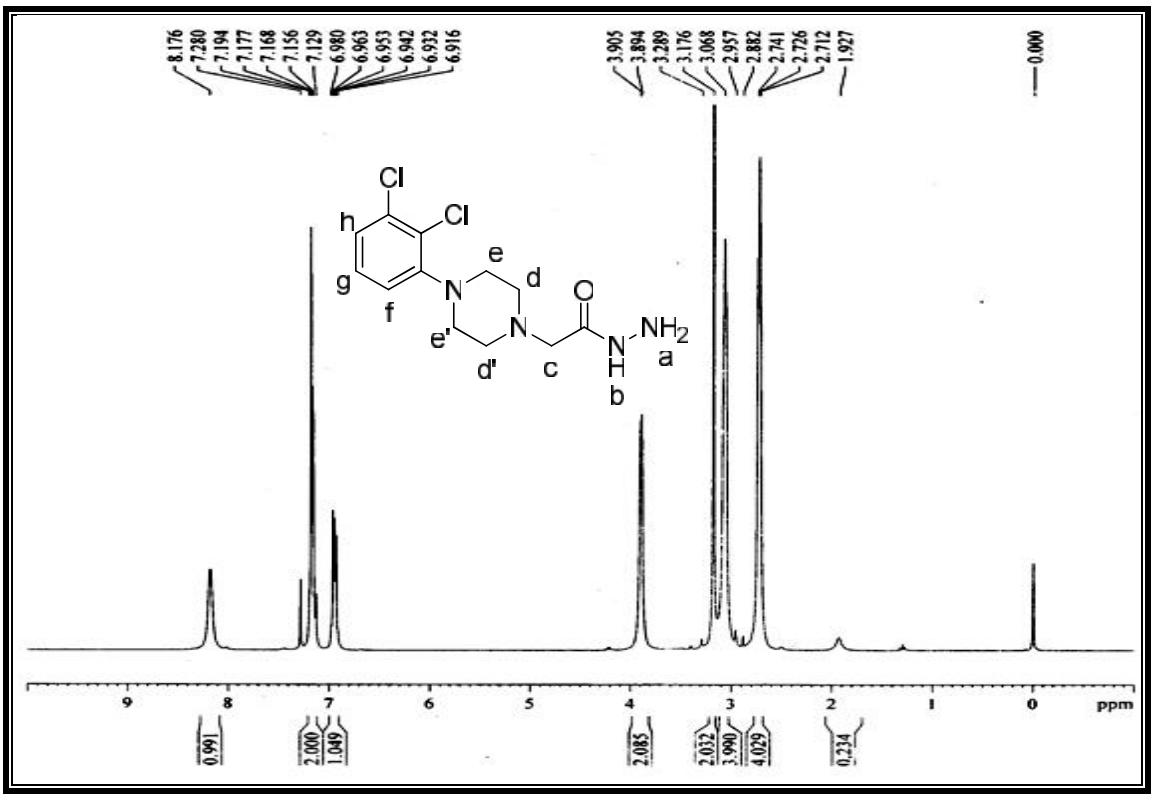
IR SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2956	2975-2920	109
	C-H str. (sym.)	2889	2880-2860	”
	C-H def. (asym.)	1450	1470-1435	”
	C-H def. (sym.)	1369	1395-1370	”
Aromatic	C-H str.	3064	3100-3000	”
	C=C	1450	1585-1480	”
	C-H i.p. def.	1112	1125-1090	”
	C-H o.o.p. def.	819	860-810	”
Carbonyl	C=O	1668	1650-1700	”
Ether	C-O-C	1236	1200-1275	”
Halide	C-Cl	779	650-850	”
Amide	-NH str.	3207	3200-3400	”
Amine	-NH str.	3292		

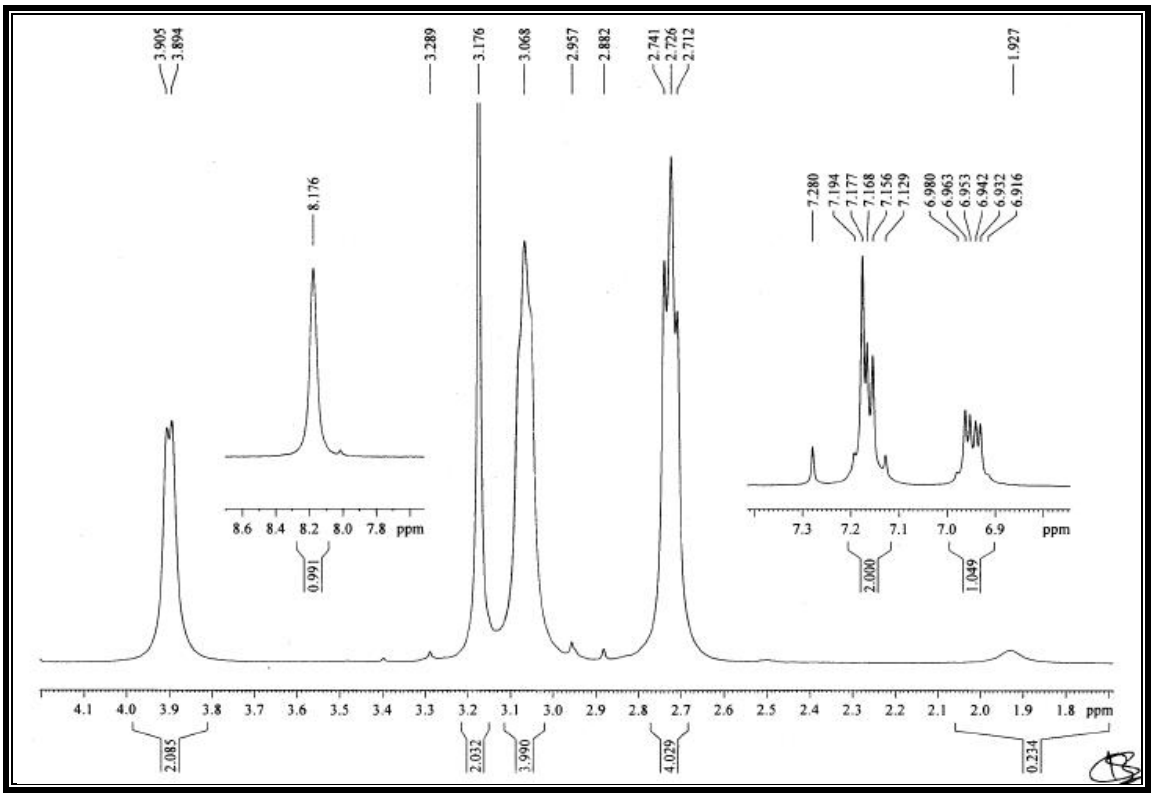
^{1}H-NMR SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE



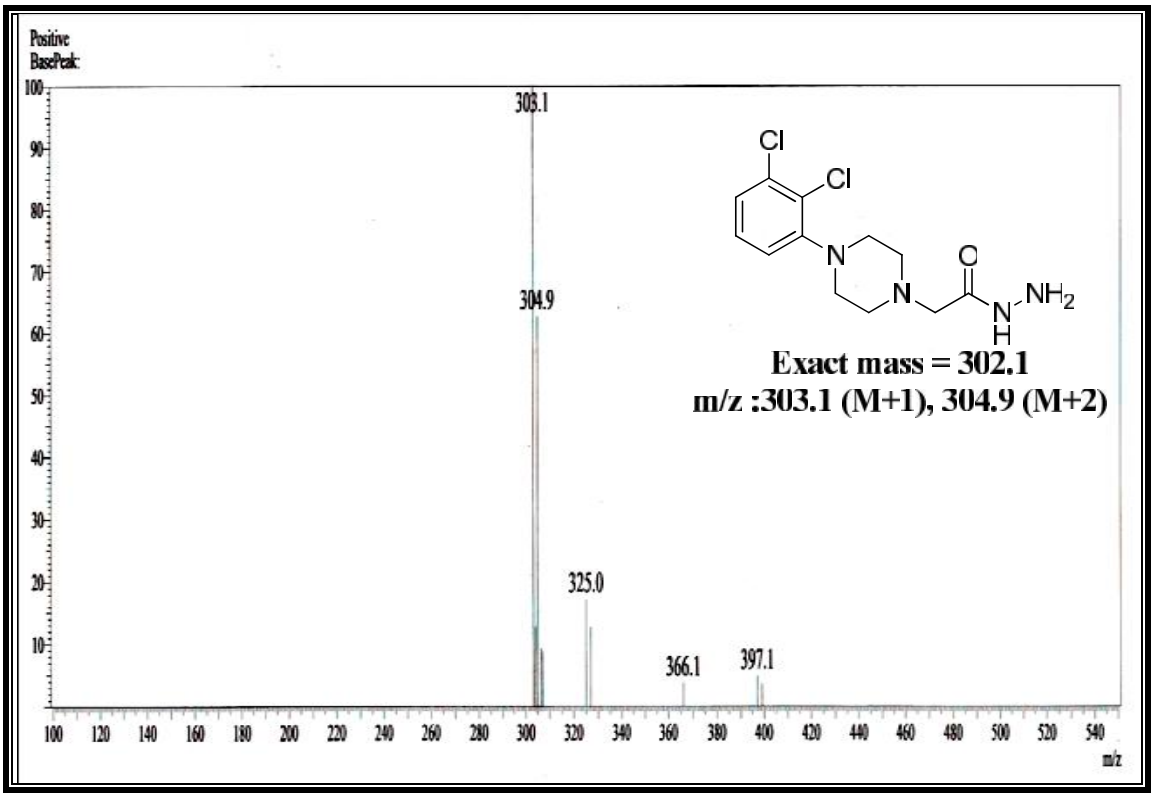
Internal Standard: TMS; Solvent: $CDCl_3$; Instrument: BRUKER Spectrometer (300 MHz)

Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	2.71-2.74	4H	triplet	-C<u>H</u>$_2$ (d,d')	-
2	3.06	4H	broad singlet	-C<u>H</u>$_2$ (e,e')	-
3	3.17	2H	singlet	-C<u>H</u>$_2$ (c)	-
4	3.89-3.90	2H	doublet	-N<u>H</u>$_2$ (a)	3.3
5	6.93-6.96	1H	double doublet	Ar-H (f)	3.0 & 6.3
6	7.12-7.19	2H	multiplet	Ar-H (g,h)	-
7	8.17	1H	broad singlet	-CO-N<u>H</u> (b)	-

EXPANDED ^{1}H-NMR SPECTRUM



MASS SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE



EXPERIMENTAL

SYNTHESIS AND CHARACTERIZATION OF 2-(4-(2,3-DICHLOROPHENYL) PIPERAZIN-1-YL)ACETOHYDRAZIDE

[A] PREPARATION OF ETHYL 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETATE

To the stirred solution of 1-(2,3-dichlorophenyl)piperazine (23.1 gm, 0.1 mol) and potassium carbonate (20.7 gm , 0.15 mol) in 100 ml acetone was added ethyl bromo acetate (18.37 gm, 0.11 mol) drop wise at 10^{o}C then stirred the reaction at room temperature for 14 hr. The progress of reaction was monitored by TLC using mobile phase ethyl acetate / hexane (6/4), after completion of the reaction solvent was removed to give residue, the residue was added to chilled water (500 ml) and stirred for overnight. The separated solid was filtered, washed with water, dried and finally crystallized from ethanol to give 24 gm cream solid. Yield: 76 %.

[B] PREPARATION OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

A mixture of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetate (3.16 gm, 0.01 mol) and hydrazine hydrate 99 % (1 gm, 0.02 mol) in 10 ml ethanol was stirred and refluxed for 8 hr. The progress of reaction was monitored by TLC using mobile phase ethyl acetate / hexane (8/2), after completion of the reaction the reaction mixture was kept in deep-freezer for overnight. The precipitated product was filtered off, washed with hexane (10 ml) and crystallized from isopropyl alcohol (IPA) to give 2.44 gm white solid. Yield: 81 %.

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Chapter-2, Part-2

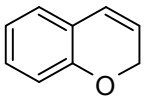
STUDIES ON CHROMENE DERIVATIVES

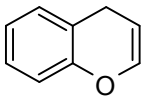
INTRODUCTION

Chromene is a polycyclic organic heterocycle in which benzene ring fused with a heterocyclic pyran ring. It is also called as benzopyran. There are two isomers of benzopyran that vary by the orientation of the fusion of the two rings compared to the oxygen, 1-benzopyran (chromene) and 2-benzopyran (isochromene) the number denotes where the oxygen atom is located by standard naphthalene like nomenclature. Its molecular formula is C_9H_8O, its molecular weight is 132.16.

The radical form of chromene is paramagnetic. The unpaired electron is delocalized over the whole chromene molecule, rendering it less reactive than one would expect otherwise, a similar example is the cyclopentadienyl radical. Commonly, chromene is encountered in the reduced state, where it is partially saturated with one hydrogen, introducing a tetrahedral methylene group in the pyran ring. Thus there are many different structural isomers are possible due to multiple possible positions of the oxygen atom and the tetrahedral carbon:

Structural isomer of chromene

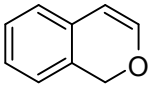


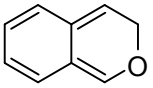


2*H*-chromene (2*H*-1-benzopyran)

4*H*-chromene (4*H*-1-benzopyran)

Structural isomer of isochromene

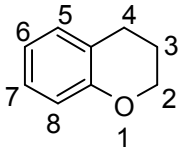




1*H*-isochromene (1*H*-2-benzopyran)

3*H*-isochromene (3*H*-2-benzopyran)

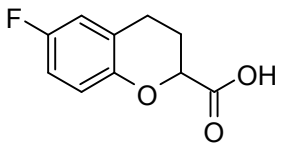
Saturated chromene is called as chroman or 3,4-dihydro-2*H*-chromene. Chroman is an aromatic bicyclic heterocycle. Its molecular formula is $C_9H_{10}O$, its molecular weight is 134.18 and chemical structure shown as under (1).



(1)

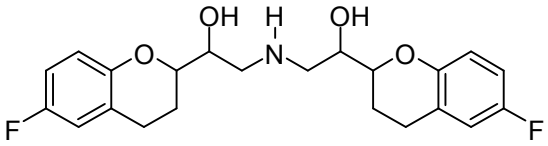
Chroman was first prepared in 1905; little interest was shown in the compound until studies on the tocopherols (Vitamin-E) began to indicate that they were derivatives of chroman. The monoalkyl chromans can be divided into two groups-one in which the alkyl substituted attached to the benzene ring and the second in which attached to the heterocyclic ring. The former can be obtained from appropriate derivatives of benzene similar to those used for the preparation of chroman. Chroman is stable to acids and oxidizing agents. It is soluble in common organic solvents[1].

Many chroman derivatives are very useful in the synthesis of drug. 6-Fluorochroman-2-carboxylic acid (2) is key intermediate in the synthesis of "Nebivolol". Nebivolol (3) is an antihypertensive drug. Nebivolol has been studied in over 3000 patients with hypertension. The use of nebivolol is contraindicated in patients with cardiogenic shock, uncontrolled heart failure, sick sinus syndrome, second and third degree heart block, asthma etc. 6-Fluorochroman-2-carboxylic acid (2) is a solid at room temperature and it is also known as nebivolol acid



(2) 6-fluorochroman-2-carboxylic acid

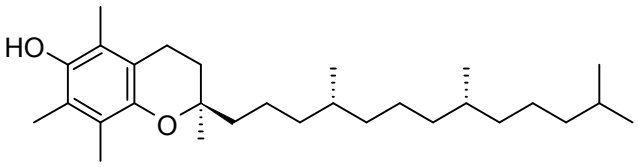
(Nebivolol acid)



(3) Nebivolol

2,2'-azanediylbis(1-(6-fluorochroman-2-yl)ethanol)

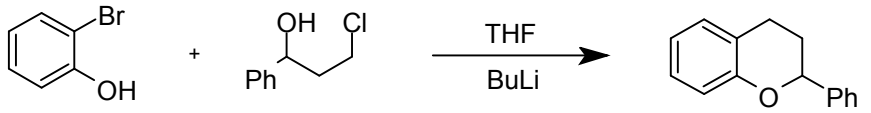
Vitamin E (tocopherol) refers to a family of eight molecules having a chromanol ring (chroman ring with an alcoholic hydroxyl group) and a 12-carbon aliphatic side chain containing two methyl groups in the middle and two more methyl groups at the end. Tocotrienols (found in high concentrations in palm oil) are many times more potent as anti-oxidants than are tocopherols (4), but they are poorly assimilated by digestion, are poorly distributed to tissues in blood and are rapidly metabolized and eliminated from the body but tocotrienols are well-absorbed by the skin and thus are well suited for use as a Vitamin E cream.



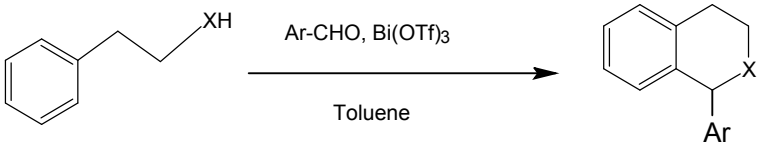
(4) α -tocopherol

SYNTHETIC ASPECT

1. K. J. Hodgetts[2] has developed a new method for 2-substituted chroman by intermolecular Mitsunobu reaction of a chiral halopropanol with 2-bromophenol.

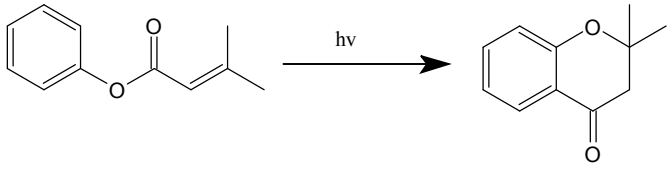


2. C. Lherbet et al .[3] have synthesized isochroman in the one pot-reaction by using different benzaldehydes and phenylethanethiol or phenyl ethanol in presence of bismuth triflate as a catalyst.

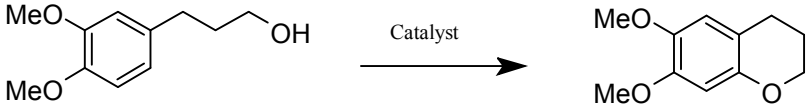


X= O,S

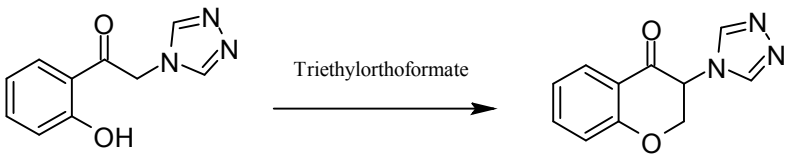
3. C. S. López et al. [4] have developed a mild and convenient one-pot photochemical synthesis of chroman-4-one derivatives.



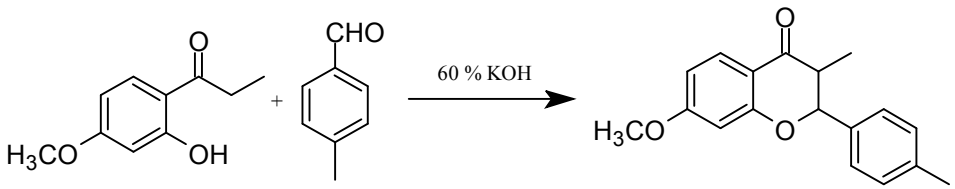
4. H. Hamamoto et al.[5] synthesized chroman by direct aromatic carbon–oxygen bond-formation reaction involving aromatic cation radical intermediates using the hypervalent iodine (III) reagent, phenyl iodine (III) bis(trifluoroacetate) (PIFA).



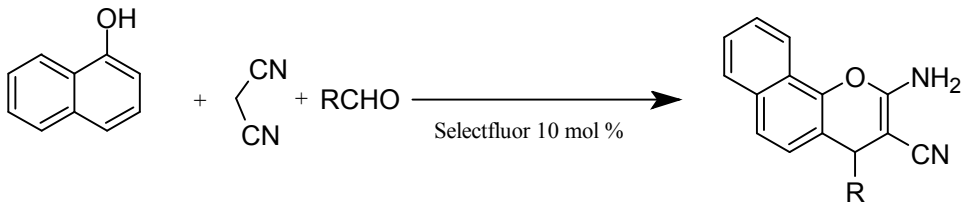
5. S. Emami et al.[6] have synthesized 3-azolyl chroman derivatives as conformationally constrained analogs.



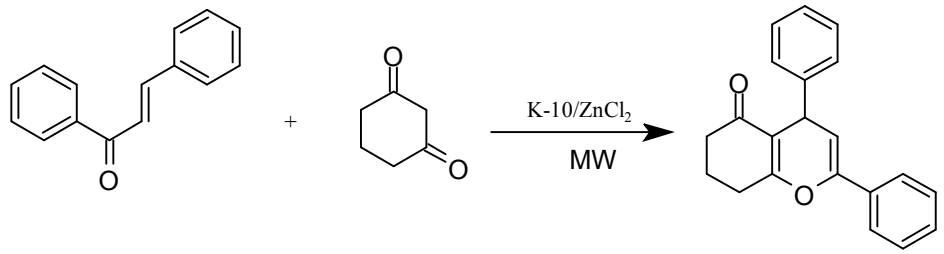
6. M. Venkati et al.[7] have synthesized chroman derivative from substituted o-hydroxy propiophenone and substituted benzaldehyde in 60% KOH.



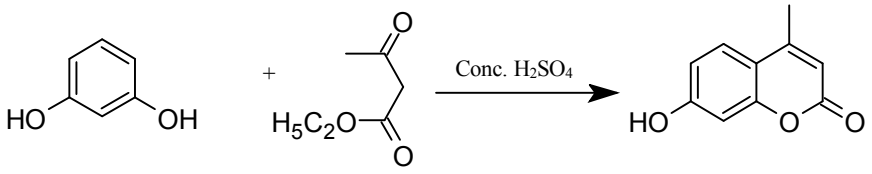
7. R. R. Karimi et al.[8] have developed simple and clean route for synthesis of 2-amino-3-cyano-2*H*-chromene derivatives.



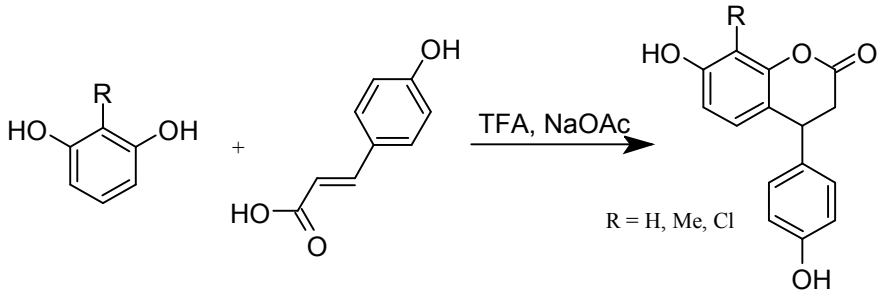
7. S. R. Sarda et al.[9] have synthesized 2,4-diphenyl-4*H*-chromen-5-one from α, β-unsaturated carbonyl compounds and 1,3-cyclohexanedione under microwave irradiation in the presence $ZnCl_2$/montmorillonite K-10.



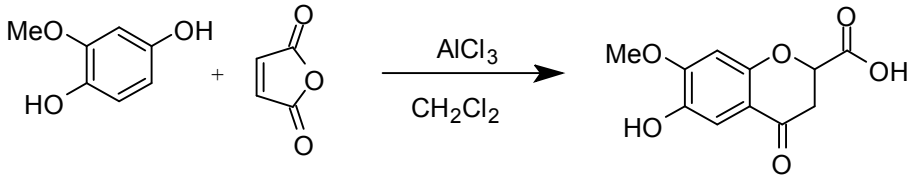
8. D. P. Kardile et al.[10] have synthesized 7-hydroxy-4-methylcoumarin from Pechmann condensation of resorcinol with ethylacetoacetate in presence of concentrated sulphuric.



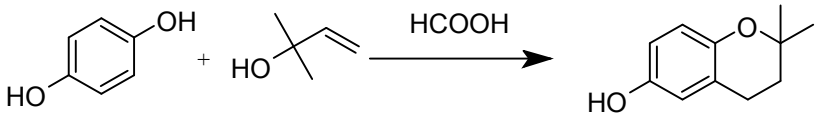
9. R. Suthunuru et al.[11] have developed highly effective, facile, one-pot regioselective synthesis of a series of 7-hydroxy-4-phenyl-chroman-2-ones.



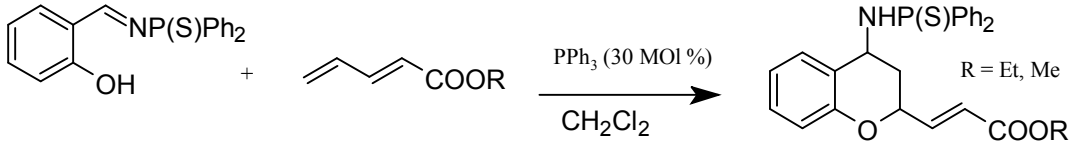
10. H. Lee et al.[12] have synthesized 6-hydroxy-7-methoxy-4-chromanone using aluminum chloride as catalyst.



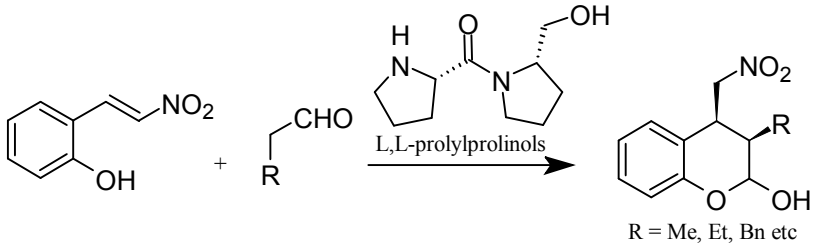
11. Q. Wang et al.[13] have synthesized 6-hydroxy chroman from condensation of 2-methyl-3-butene-2-ol and substituted phenol in the presence of formic acid.



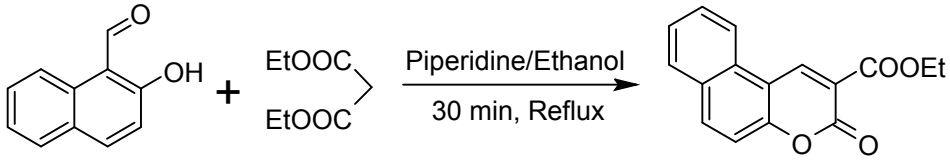
12. X. Meng et al.[14] have synthesized highly functionalized chroman derivatives, reaction was catalyzed by triphenylphosphine.



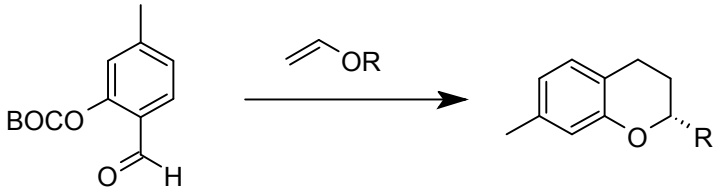
13. D. Lu et al.[15] have synthesized 2-hydroxy-3-alkyl-4-nitromethyl-chroman derivative by the michael type reaction between aliphatic aldehydes and (*E*)-2-(2-nitrovinyl)phenols.



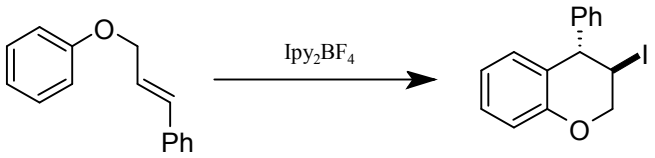
14. Rajesha et al.[16] synthesized ethyl 3-oxo-3*H*-benzo[*f*]chromene-2-carboxylate in the presence of piperidine in dioxane from 2-hydroxynaphthalene-1-carboxlate



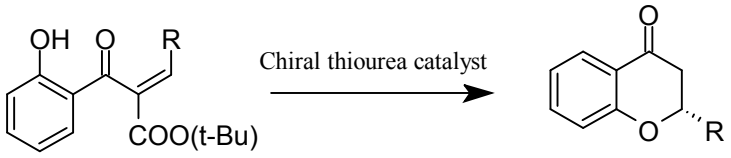
15. C. Selenski et al.[17] have synthesized chroman derivatives as like natural molecule of (+)-mimosifoliol.



16. J. Barluenga et al.[18] have synthesized chroman derivatives by the reaction of different ally phenyl ethers with Ipy_2BF_4.



17. M. M. Biddle et al.[19] have synthesized flavanones and chromanone using bifunctional thiourea catalysts promote an asymmetric oxo-conjugate addition to a β-ketoester alkylidene.

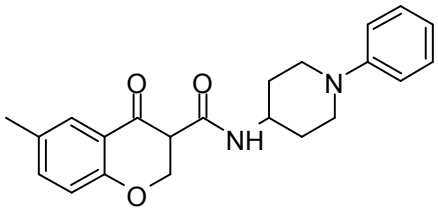


THERAPEUTIC IMPORTANCE

Croman is the important heterocyclic compound. The number of bioactive compounds containing croman ring system is so vast that the complete range of their biological activities can be hardly classified[20-22], however

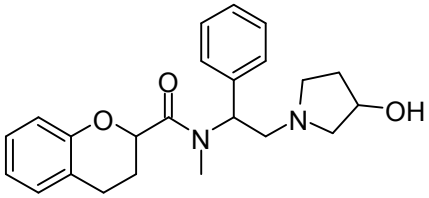
1. Antifungal[23]
2. Antibacterial[24,25]
3. Antioxidant [26]
4. Anti HIV[27]
5. Antiarrythmic[28]
6. antiepileptic agents[29-31]
7. antihypertensive[32]
8. Antiviral[33]
9. Antiallergic[34]
10. Anti-inflammatory[35]
11. Antitumor[36]
12. Antitubercular[37]
13. Antidiabetic[38]
14. Hepatoprotective agents[39]
15. Antiulcer activity[40]

M.C.Patel et al.[41] have synthesized some novel chroman derivative (5) and studied their antibacterial and antifungal activities, using the *E. coli, P. aeruginosa, S. aureus, S. Pyogenus and C. albicans.*



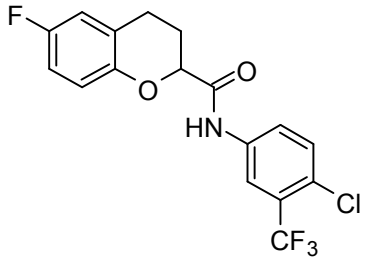
(5)

G. Hua et al.[42] have synthesized chroman based derivative (6) as a potent and highly selective kappa opioid receptor agonists.



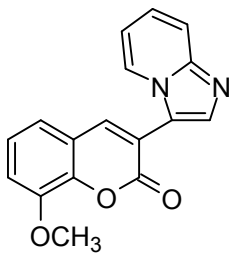
(6)

B. S. Priya et al.[43] have synthesized 6-fluoro-chroman-2-carboxamides (7). These molecules were evaluated for their invitro efficacy as antimicrobials by disc diffusion and microdilution method against pathogenic strains such as *Bacillus substilis*, *Escherichia coli, Pseudomonas fluorescens, Xanthomonas campestris pvs, X. oryzae, Aspergillus niger, A. flavus, Fusarium oxysporum, Trichoderma species, F. monaliforme,* and *Penicillum species.*



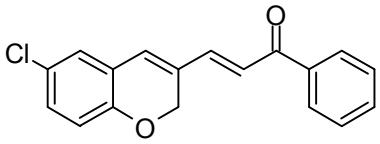
(7)

P.V.Kumar et al.[44] have synthesized 3-indolizin-3-yl-chromen-2-one (8) as a antitubercular, antiviral and anticancer activities.



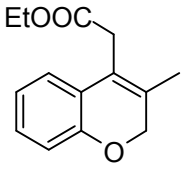
(8)

Z. Nazarian et al.[45] have synthesized a chalconoids derivative containing a 6-chloro-2*H*-chromen-3-yl (9) and showed cytotoxicity assessment against mouse peritoneal macrophage cells.



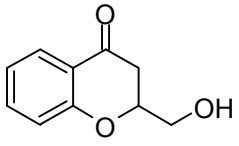
(9)

S. Gowrisankar et al.[46] have synthesized 4-substituted 3-*exo*-methylenechroman derivatives (10) and evaluated as a antimicrobial agents.



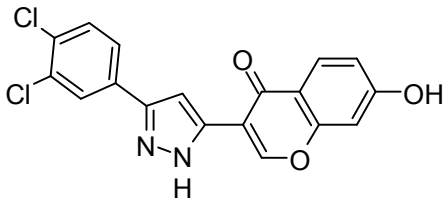
(10)

J. G. Kanga et al.[47] have isolated 2-hydroxymethyl-chroman-4-one (11) which exhibited good activities against phytopathogen such as *Pythium ultimum*, *Phytophthora capsici* and *Sclerotinia sclerotiorum*.



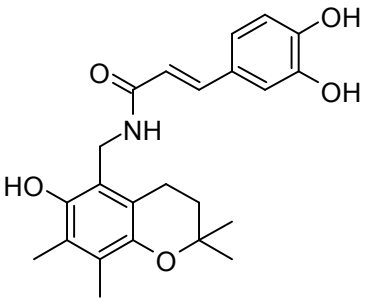
(11)

K.Hatzade et al.[48] have synthesized 7-hydroxy-3-pyrazolyl-chroman-4-ones derivaive (12) and evaluated for their in vitro antimicrobial and antioxidant activity.



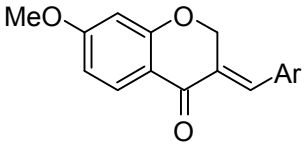
(12)

M. Koufaki et al.[49] have synthesized 6-hydoxy-5-substituted chroman derivatives (13) and evaluated their activity against oxidative Stress Induced Cellular Damage.



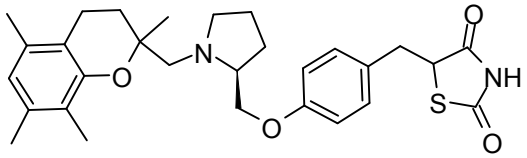
(13)

T. Shankar et al.[50] have synthesized 3-arylidene-7-methoxy-4-cromanones (14) and evaluated their anti-inflamatory activity.



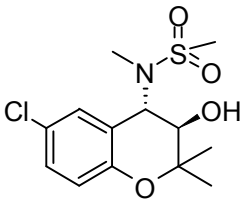
(14)

K. A. Reddy et al.[51] have synthesized chroman derivatives having 2,4-thiazolidinone moiety (15) and evaluated for hypolipidemic activities.



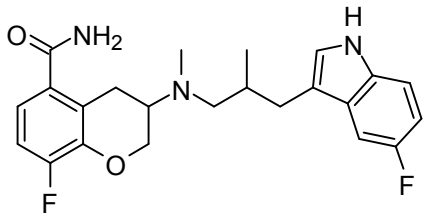
(15)

U. Gerlach et al.[52] have synthesized various methanesulfonamide derivative (16) containing chroman moiety for development as an antiarrhythmic drug.



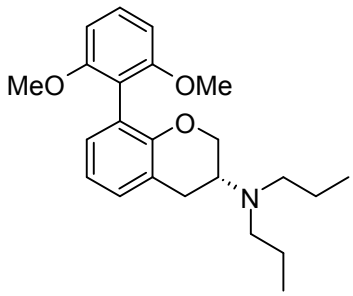
(16)

N. T. Hatzenbuhler et al.[53] have worked on combining 3-aminochroman scaffold (having affinity for 5-HT_{1A} receptor) and 5-fluoroindole analogues(having affinity for 5-HT reputake site) linked through a common basic nitrogen via an alkyl chain attached at the 1- or 3-position of the indole evaluated for dual affinity at both the 5-HT reuptake site and the 5-HT_{1A} receptor.



(17)

P. Holmberg et al.[54] have synthesized novel 2-aminotetralin and 3-aminochroman derivatives (18) as selective serotonin 5-HT_7 receptor agonists and antagonists.



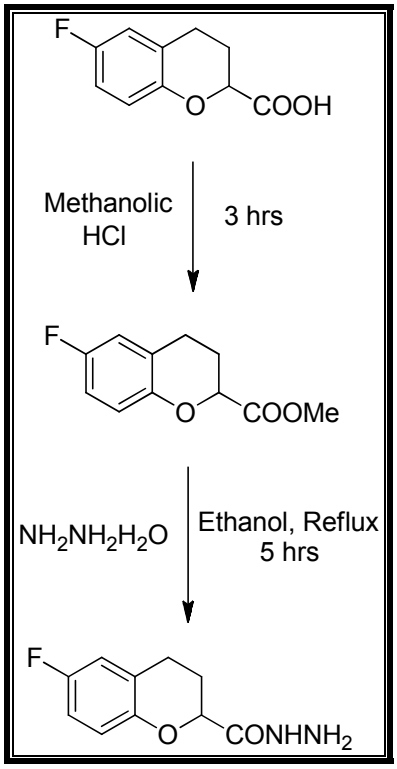
(18)

Thus the important role displayed by chromene and its derivatives for various therapeutic and biological activities prompted us to synthesize some Schiff base, Oxadiazole and Aryl amide derivatives bearing chromene moiety in order to achive compounds having better biological activities.

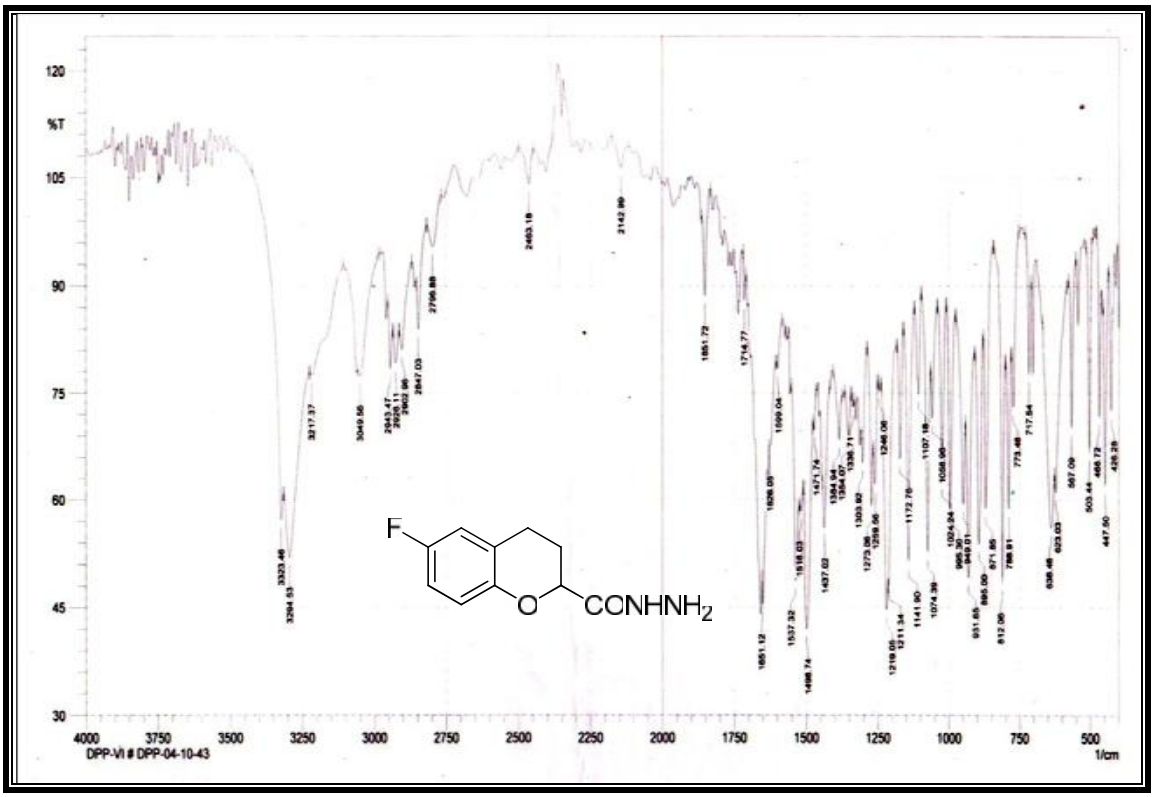
SECTION-I

SYNTHESIS AND CHARACTERIZATION OF 6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

REACTION SCHEME



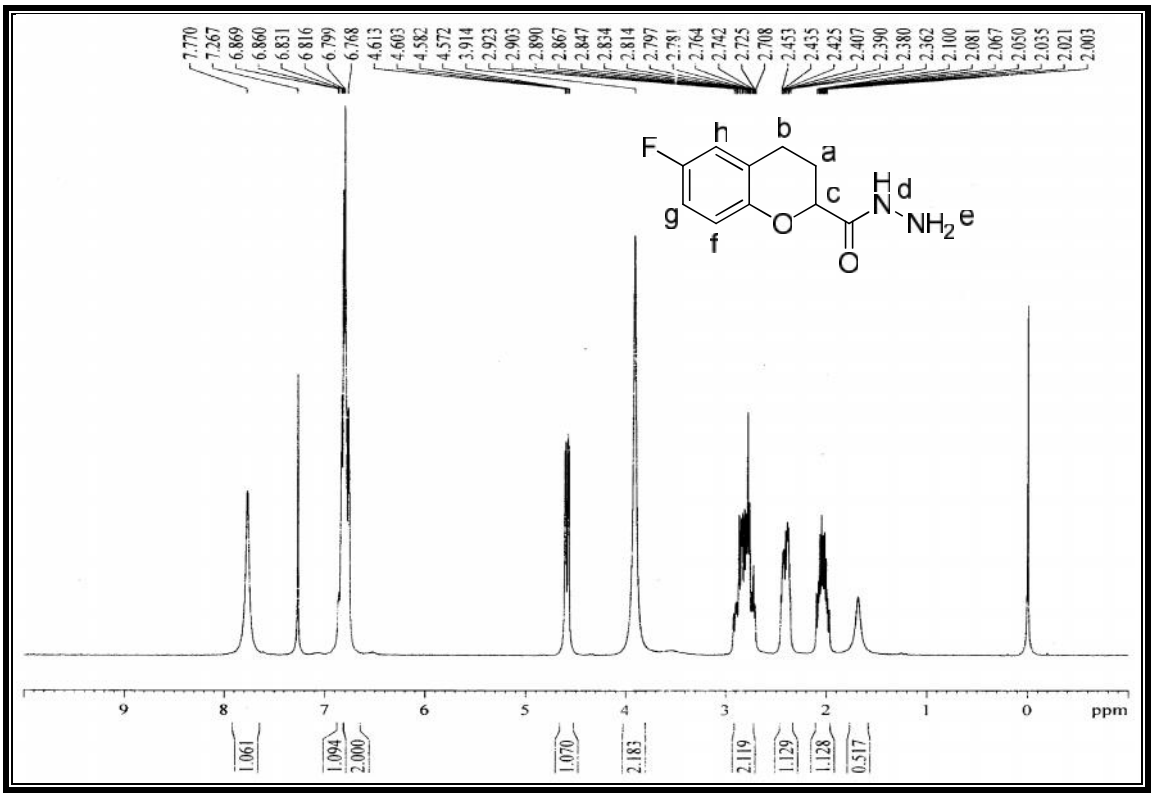
IR SPECTRUM OF 6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2943	2975-2920	55
	C-H str. (sym.)	2847	2880-2860	”
	C-H def. (asym.)	1437	1470-1435	”
	C-H def. (sym.)	1384	1395-1370	”
Aromatic, Hetocycle	C-H str.	3049	3100-3000	”
	C=C str	1498	1585-1480	”
	C-H i.p. def.	1074	1125-1090	”
Ether	C-O-C	1219	1275-1200	”
Amide	C=O	1651	1700-1650	”
Amine	-NH str.(secondry)	3217	3320-3140	”
	-NH str.(primary)	3294, 3323	3400-3200	”

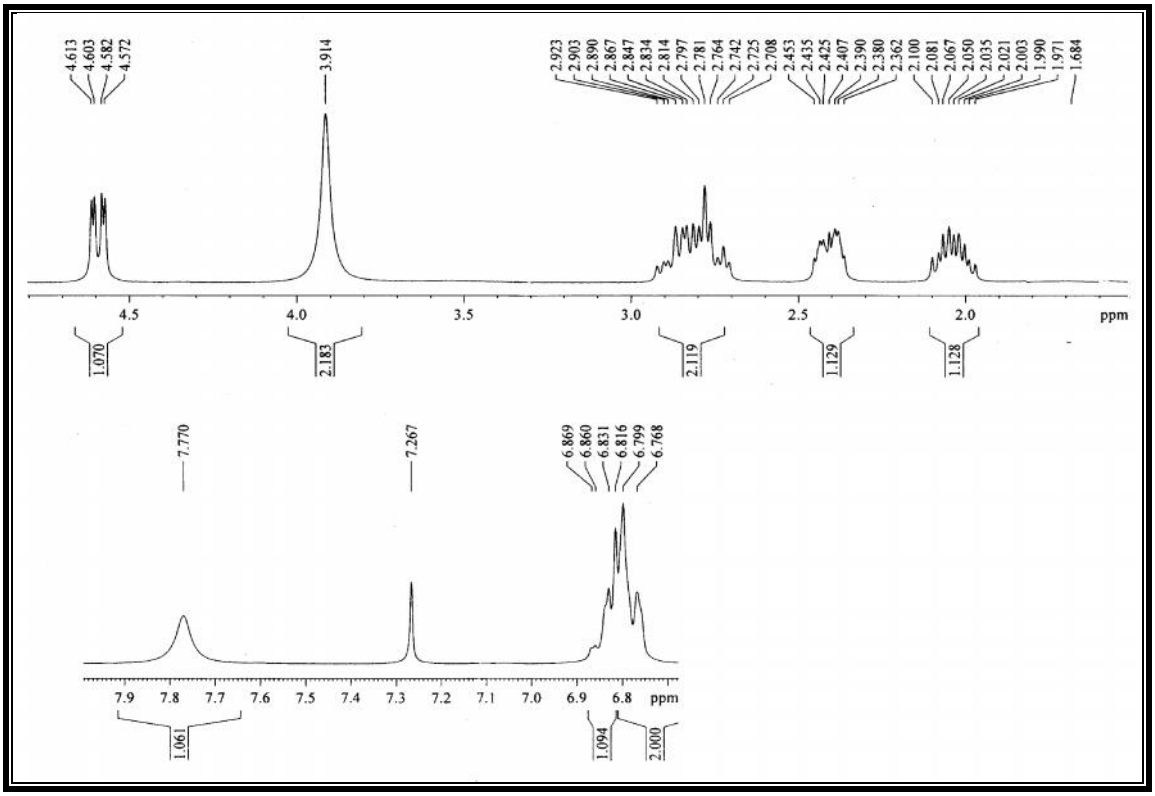
^{1}H-NMR SPECTRUM OF 6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



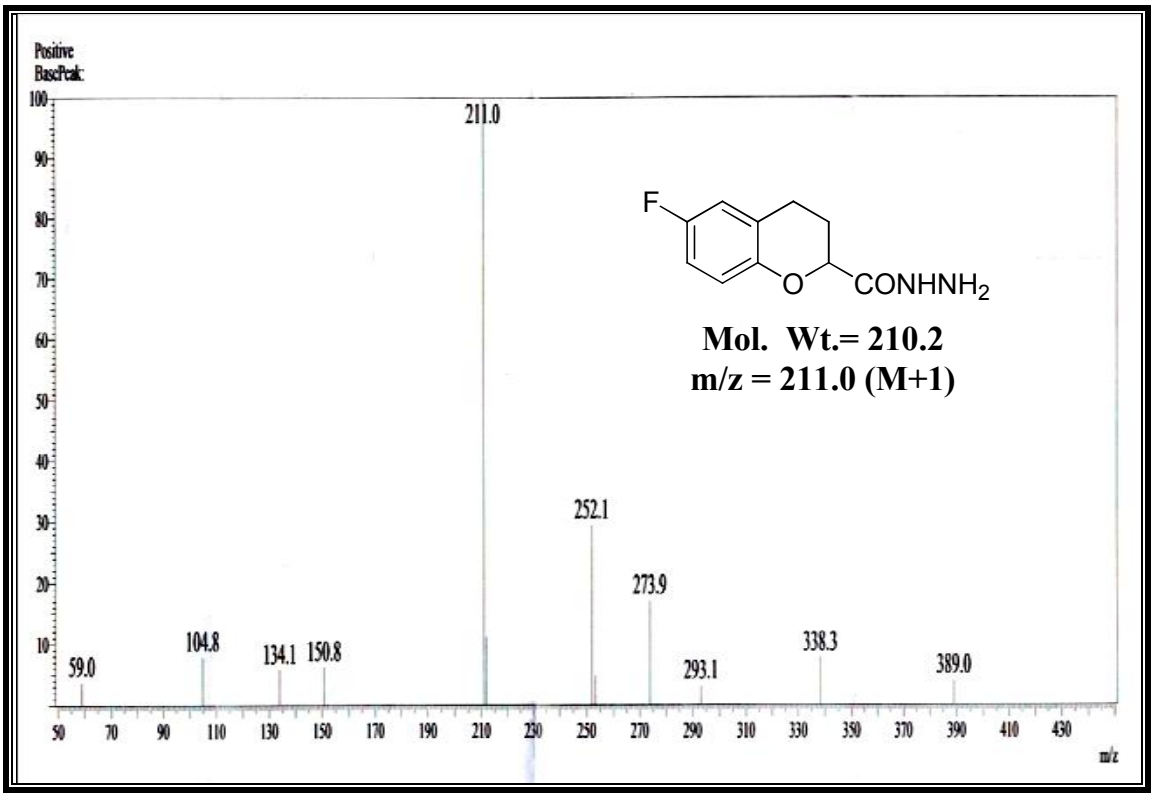
Internal Standard: TMS; Solvent: $CDCl_3$ Instrument: BRUKER Spectrometer (300MHz)

Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	2.00-2.10	1H	multiplet	-CH (a)	-
2	2.36-2.43	1H	multiplet	-CH (a)	-
3	2.70-2.92	2H	multiplet	-CH (b)	-
4	3.91	2H	broad singlet	-NH_2 (e)	-
5	4.57-4.67	1H	double doublet	-CH (c)	3.0 & 9.3
6	6.76-6.86	3H	multiplet	Ar-H (f,g,h)	-
7	7.77	1H	broad singlet	-CO-NH (d)	-

EXPANDED ^{1}H-NMR SPECTRUM



MASS SPECTRUM OF 6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



EXPERIMENTAL

SYNTHESIS AND CHARACTERIZATION OF 6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

[A] PREPARATION OF METHYL 6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOXYLATE

A mixture of 6-fluoro-3,4-dihydro-2*H*-chromene-2-carboxylic acid (0.1 mole, 19.6 gm) and solution of 10 % HCl (59.1 ml, 3 V/W) in methanol was stirred at 30 ^{o}C for 3 hr. The progress of the reaction was monitored by TLC using mobile phase Methanol/chloroform (9.5/0.5), after completion of the reaction solvent was removed under reduced pressure to give an oily product (21 gm) which was directly used in the next step.

[B] PREPARATION OF 6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

A mixture of methyl 6-fluoro-3,4-dihydro-2*H*-chromene-2-carboxylate (21.0 gm, 0.1 mole) and hydrazine hydrate 99 % (10 gm, 0.2 mole) in 63 ml ethanol was stirred and refluxed for 5 hr. The progress of reaction was monitored by TLC using mobile phase Methanol/chloroform (9.5/0.5), after completion the reaction mixture was then kept in deep-freezer for overnight. The precipitated product was filtered off, washed with hexane (100 ml) and crystallized from isopropyl alcohol (IPA) to give 14.91 gm off white solid. Yield: 71 %.

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3. C. Lherbet, D. Soupaya, C. B. Dehoux, C. André, C. Blonski, P. Hoffmann; *Tet. Lett.,* **49**, 5449-5451 (2008).
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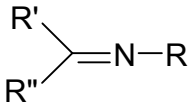
Chapter-3

STUDIES ON SCHIFF BASE DERIVATIVES

INTRODUCTION

The condensation of primary amines with carbonyl compounds was first reported by Schiff [1], and the condensation products are often referred to as Schiff bases. The general structure of these bases is



(1)

Where R, R' and R" are H or alkyl or cycloalkyl or aryl or heterocycle, which may be in derived form. This condensation reaction, along with the chemical and physical properties of Schiff bases have been reviewed.[2-4] Schiff bases are also known as Schiff's bases or azo methines or imines, they are well known intermediate for the preparation of azetidinone, thiazolidinone and many other pharmaceutically important entities. These are the compounds containing characteristic >C=N– group. Various studies have been shown that the >C=N- group has considerable biological importance.

In recent years, there has been an increasing interest in the design and development of Schiff base derivatives because they are associated with antibacterial, antifungal and antitubercular activities and have diverse biological activities [5]. Schiff bases are known to be useful in perfumery[6,7], as corrosion inhibitor[8,9], as complexing agents[10,11] and as intermediate in many reactions[12-16]. They are used in optical and electrochemical sensors, as well as in various chromatographic methods, to enable detection of enhance selectivity and sensitivity[17-19]. In literature, some other applications of schiff bases have also been reported in various fields [20-24]. Some of these compounds and their metal complexes have also been used in the preparation of various medicines[25-29]. Further, many workers[30-37] reported a wide range of biological activities of various schiff bases. Besides, several schiff bases have been reported to possess remarkable antibacterial[38-42], antifungal[43-48], anticancer[49-53], anti HIV[54-56], anti-inflammatory[57-60], antiparasitic[61,62] and diuretic[63] activities.

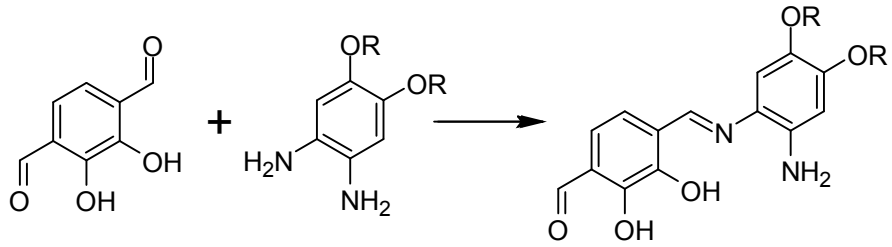
Schiff's bases are obtained mainly by warming the aldehyde & aromatic amine together. However, it is more convenient to work in a solvent such as alcohol, dilute acetic acid or glacial acetic acid. Some time the reaction is aided by trace of acid in other cases the hydrochloride of the amines can be used in the synthesis. In general Schiff's bases do not react further with either of the reagents used in their preparation as

do most of the other types of simple intermediates. Synthetic Schiff's base derivatives contribute in huge libraries owing to their wide applicability in different fields.

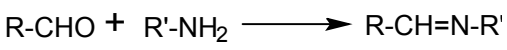
SYNTHETIC ASPECT

Different methods for the preparation of azomethine derivatives documented in literature are described as under.

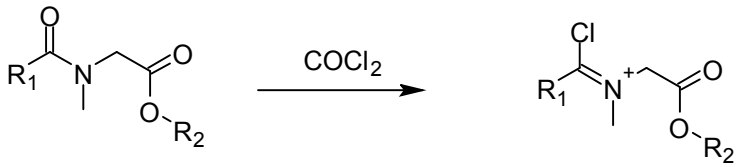
1. A. J. Gallant et. al.[64] have prepared schiff's bases by condensation of equimolar quantity of 3,6-diformyl catechol and substituted o-phenylene diamine.



2. General account of the summary of reaction of aldehydes with amine (aromatic or aliphatic) has been reviewed by M. S. Murray.[65]

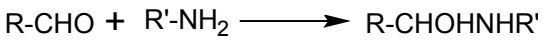


3. A new one pot procedure for the generation of azomethine has been investigated by R. J. Anderson and co-workers.[66]

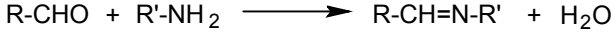


4. Strache[67] and Van Alphen[68] have prepared imine involves in two steps.

   (a) Add. of the amine to the aldehyde gives aldol, which are rarely capable of isolation.

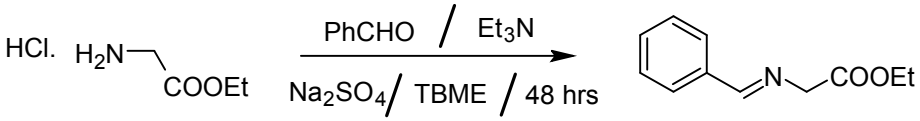


   (b) The loss of water to give an imine (azomethine), this corresponds to the "crotonaldehyde stage" of the aldol condensation.



5. Oddo and Tognacchini[69] have introduced the comparative rates of formation of Schiff's base from aromatic amines and aldehydes using a cryscopic method follow the course of reaction.

6. P. L. Beaulieu and co-workers[70] have synthesized (*E*)-*N*-phenyl methylene glycine ethyl ester by the cyclocondensation of glycine ethyl ester hydrochloride with benzaldehyde in methyl t-butyl ether (MTBE) in presence of anhydrous Na_2SO_4 and triethylamine



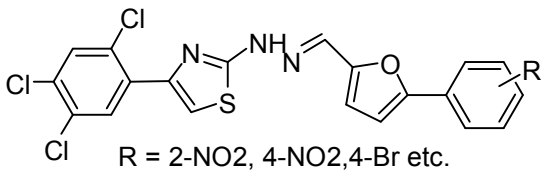
THERAPEUTIC IMPORTANCE

Literature survey reveals that various schiff's bases reported as potential drugs and are known to possess broad spectrum of biological activites.

1. Antiviral[71]
2. Antifungal[72]
3. Antiparasitic[73]
4. Antibacterial[74]
5. Antipyretic[75]
6. Antiinflammatory[76]
7. Antitubercular[77]

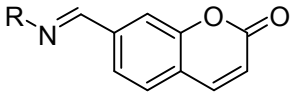
Pandey Taruna et al.[78] prepared azomethines and their boron complexes and screened for their antifungal and antibacterial properties. It is evident that azomethines alone were quite toxic but their activity increased after complexation. Omar et al.[79] have determined cyclocondensation of azomethines having good antischistosomal activity. Praveen M. and co-workers[80] have synthesized a novel class of acetyl ferrocene derived from Schiff bases having antimicrobial activity. Ram Tilak et al.[81] have synthesized some Schiff's bases, thiazolidinones 4-triazolines, and formazones of 2-chloro phenothiazines and screened as anti-inflamatory agent. The thiazolidinones showed promising anti-inflammatory activity.

Ravindra V. Chambhare et al.[82] have prepared some azomethines and tested for their antimicrobial activity. B. Shivarama Holla et. al.[83] have synthesized azomethines (2) having antibacterial and anti-inflammatory activity.

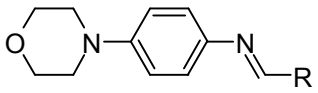


(2)

R. H. Mehta et al.[84] have synthesized schiff's base derivatives (3) containing coumarin moiety and examined for their antibacterial activity. A. K. Khalafallah and M. E. Hassan[85] have prepared some styryl schiff base derivatives as potential antibacterial and antifungal agent. P. Perumal[86] have synthesized some azomethine derivatives (4) having good antibacterial activity.



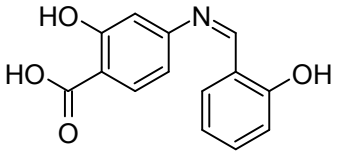
(3)



(4)

M. D. Deshmukh and A. G. Doshi[87] have prepared some schiff's bases having good antimicrobial activity against test organism *S. aureus, E. coli, S. dysenteridse* and *S. typhi*. Wang et al.[88] have synthesized diazomethines having good plant hormone activity. S. Castellano et. al.[89] have prepared azomethine derivatives and evaluated in vitro against several pathogenic fungi responsible for human disease. Ali yusuf et al.[90] have synthesized some schiff's base derivatives of glucose containing acetylenic bond. The prepared schiff bases were tested for their bactericidal activity against *E. coli* and *S. aureus*.

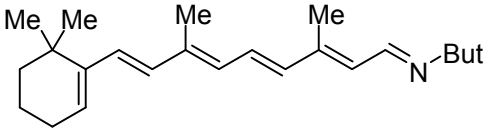
Smalders et. al.[91] reported Schiff's base as potential antitumor agents. Sharaf El-Din and Nabaweyal[92] have synthesized some Schiff's base derivatives (5) having good antibacterial activity. Chohan et. al.[93] have synthesized Schiff's bases, which have been screened and compared for their antibacterial action against bacterial species *E. coli*, *P. aeruginose* and *K. pneumoniase*.



R=3,4-OH, 3,4-NO_2

(5)

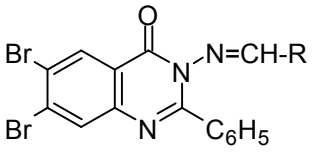
Joydip Das et. al.[94] reported trans-N-refinylidene-n-butylamine (6) which found stabillized in liposomes of phophatidylcholine. The rate of formation of the Schiff's base is found to decrease with increasing cholesteral concentration in the membrane. V. M. Patel et.al.[95] synthesized some new Schiff's bases having good antibacterial activity.



(6)

A. Cascaval et al.[96] have synthesized azomethines, which have good analgesic and antipyretic properties. S. N. Pandeya et al.[97] have synthesized schiff bases showed good activity against *Vibrio cholerae non-o.*, *Shigella boydii, Salmonella typhi, Enterococcus faecalis* and *Edwaredsiella torla* with MIC in the rang of 10-25 µg/ml. Some compounds were found to be active against *Vibro cholerae non-o*. and *Salmonella typhi.* K. N. Venugopal et al.[98] have synthesized schiff base of 4-hydroxy-6-carboxyhaydrazino benzothiophene analog with different substituted aldehydes and determined pharmacological study. Ergenc and coworkers[99] have synthesized azomethine derivatives having antifungal activity. B. Yadav and S. S. Sangapure[100] have synthesized some azomethines and tested for their biological activity.

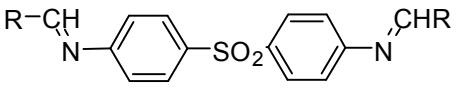
Perumal Panneerselvam et al.[101] have been synthesized some novel Schiff's bases by condensation of 3-amino-6,8-dibromo-2-phenylquinazolin-4(3*H*)-ones with different aromatic aldehydes via cyclized intermediate 6,8-dibromo-2-phenyl benzoxazin-4-one. The synthesized compounds 3-(benzylideneamino)-6,8-dibromo-2-phenylquinazolin-4(3*H*)-one (7) were screened for anti-bacterial and anti-fungal activity by paper disc diffusion technique.



R=Aryl

(7)

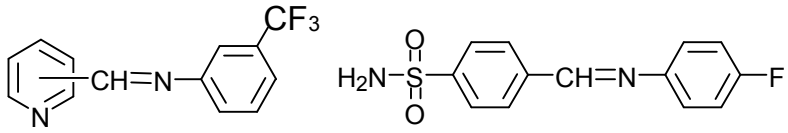
S. J. Wadher et. al.[102] have been synthesized schiff base derivatives (8) having antibacterial and anti-fungal activity.



R=Aryl

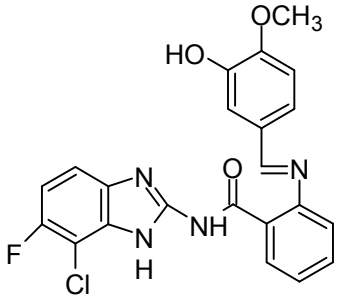
(8)

J. R. Dimmock et. Al.[103] have reported Schiff's bases as Cytotoxic agents. A. Adnan et. al.[104] have synthesized Schiff's bases possessing significant antibacterial and antifungal activity. Iana Vazzana et. al[105] described sets of Schiff bases (9) and (10) (diaryl- and arylheteroaryl azomethines) endowed with strong and long lasting antiinflammatory activity against the rat hind paw edema induced by carrageenan.



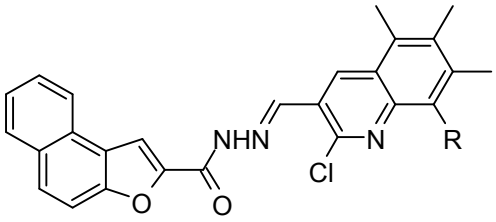
(9) (10)

Vijay Kumar. M. M. J. et. al.[106] have synthesized some Schiff bases (11) containing different functional groups and check antimicrobial activity against gram-positive and gram-negative bacteria.



(11)

Gundibasappa K. Nagaraja et. al.[107] have been synthesized some Schiff bases (12) and screened for their antibacterial and antifungal activities.



(12)

Work done from our laboratory

K. M. Thaker et al.[108] have prepared some schiff bases bearing benzo[*b*]thiophene nucleus and tested for their antitubercular and antimicrobial activity. S. L. Vasoya[109] reported facile synthesis of some new azomethines bearing benzo[*b*]thiophene nucleus as a potent biological active agent.

T. K. Dave have been reported synthesis and pharmacological study of Mannich bases of 4-amino-3-mercapto-5-pyridin-3'-yl-[1,2,4]-triazole[110] and schiff base[111] bearing nicotinic acid nucleus with antitubercular and antimicrobial evaluation.

Looking to the interesting properties of Schiff's bases, we have synthesized some new Schiff's bases, which have been described as under.

SECTION-I: SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-ARYL METHYLIDENE-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

SECTION-II: SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-(1-ARYL ETHYLIDENE)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

SECTION-III: SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-ARYL METHYLIDENE-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

SECTION-IV: SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-(1-ARYL ETHYLIDENE)-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRZIDE

SECTION-I

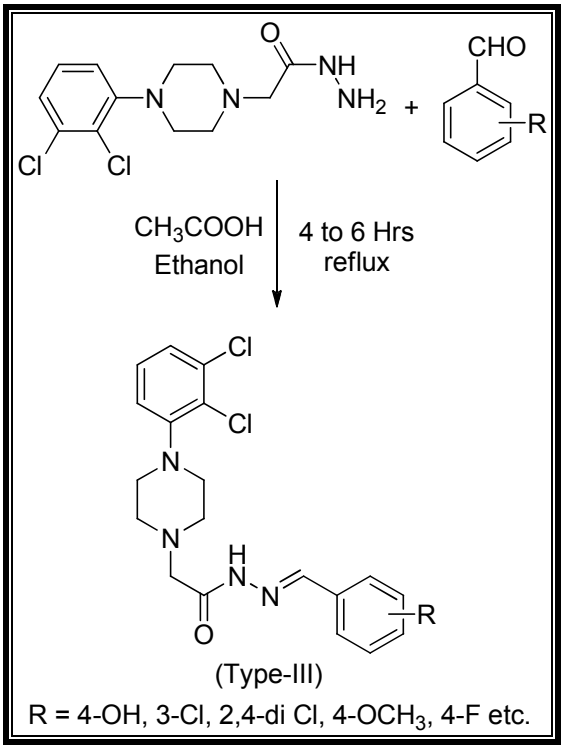
SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-ARYLMETHYLIDENE-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

Looking to the interesting properties of azomethines, with an intension to synthesizing better therapeutic agents, azomethine derivatives of Type-(III) have been synthesized by the condensation of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide with different aromatic aldehydes in order to study their biodynamic behavior.

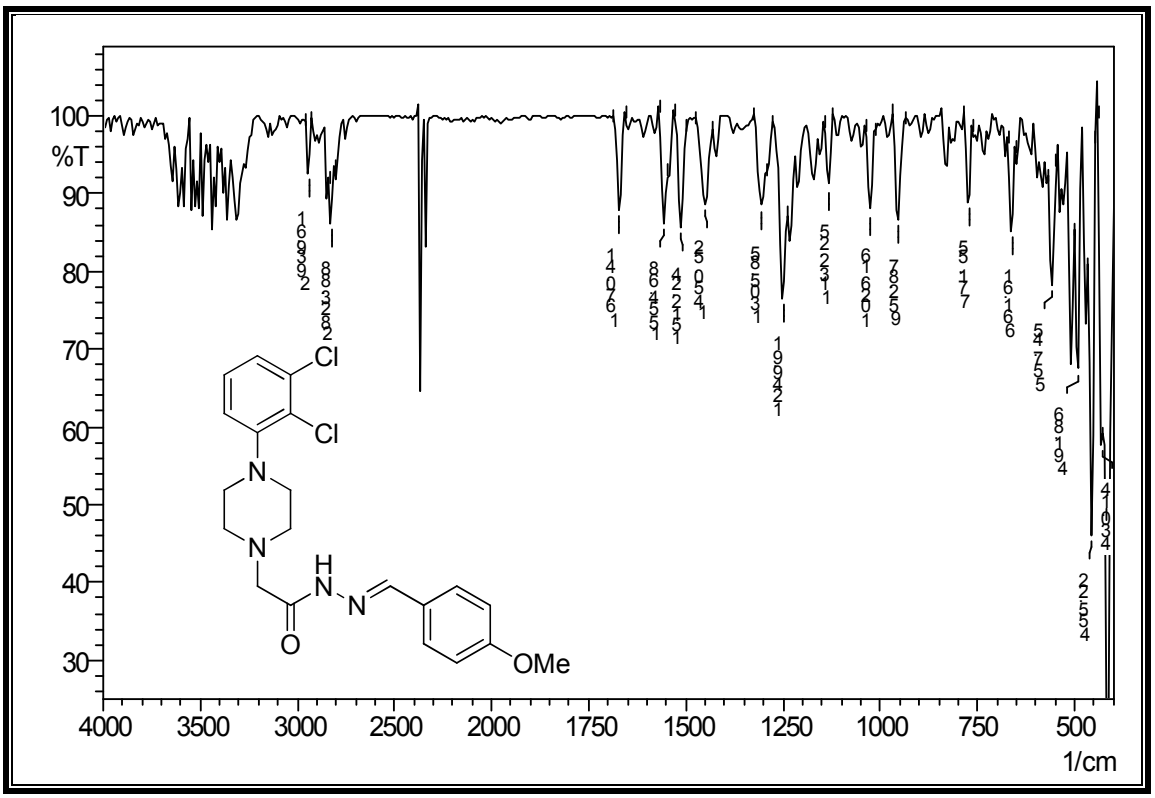
The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

REACTION SCHEME



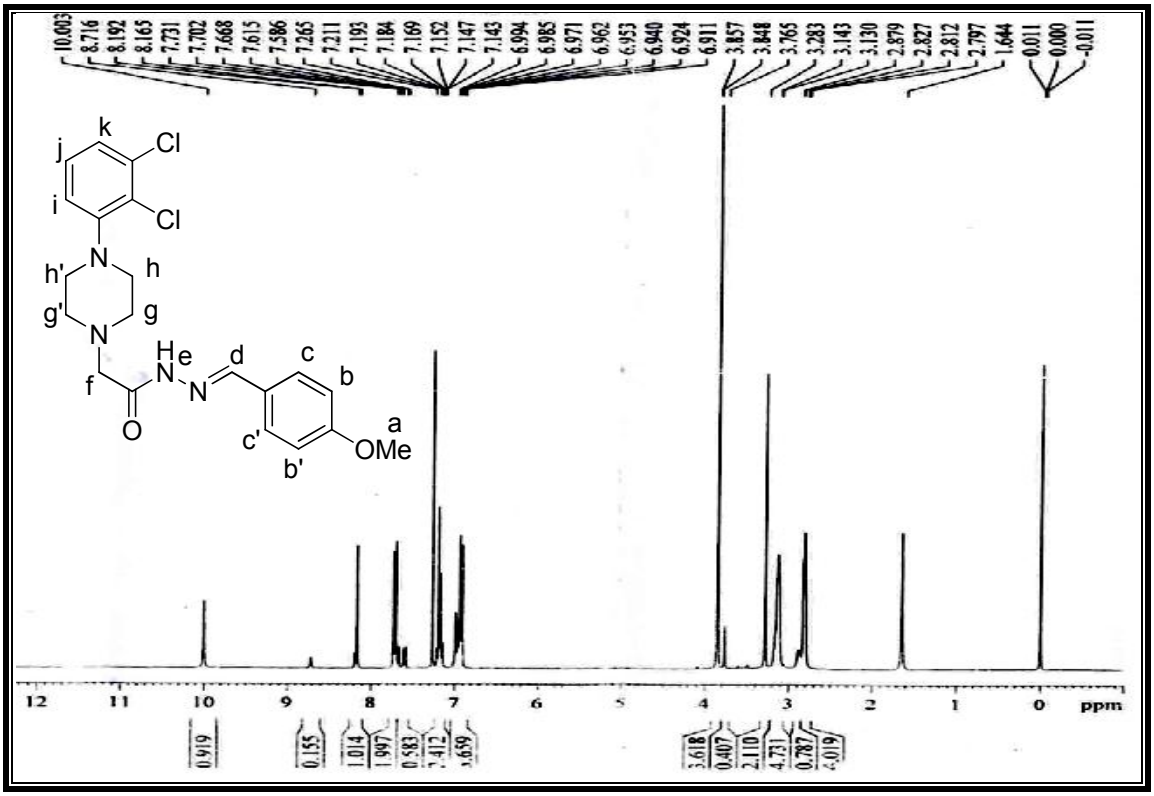
IR SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*'-((4-METHOXYPHENYL)METHYLIDENE)ACETOHYDRAZIDE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2939	2975-2920	112
	C-H str. (sym.)	2823	2880-2860	”
	C-H def. (asym.)	1450	1470-1435	”
	C-H def. (sym.)	1305	1395-1370	”
Aromatic	C-H str.	3110	3100-3000	”
	C=C	1512	1585-1480	”
	C-H i.p. def.	1132	1125-1090	”
	C-H o.o.p. def.	840	860-810	”
Azomethine	N=C str.	1554	1650-1580	”
	C-N str.	1249	1350-1200	”
Carbonyl	C=O	1670	1700-1650	”
Ether (-OMe)	C-O-C	1230	1275-1200	”
Halide	C-Cl	771	850-650	”

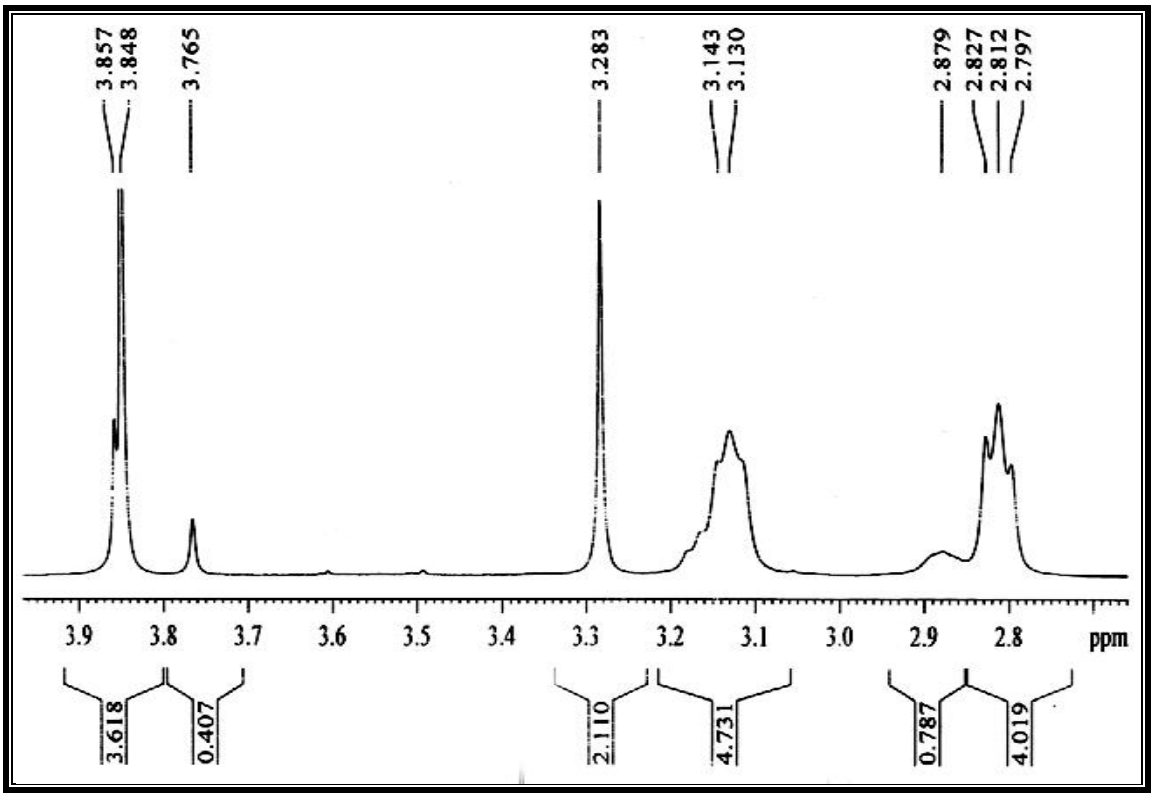
^{1}H-NMR SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*'-((4-METHOXYPHENYL)METHYLIDENE)ACETOHYDRAZIDE

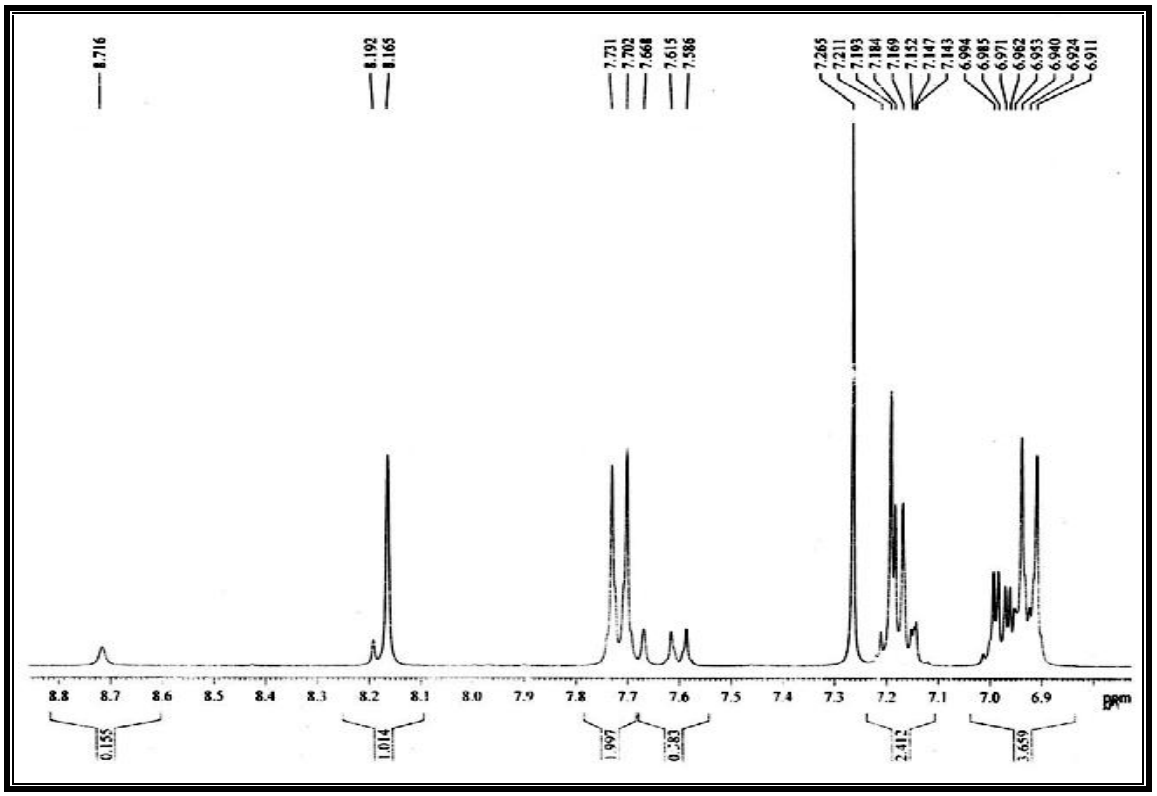


Internal Standard: TMS; Solvent: $CDCl_3$ Instrument: BRUKER Spectrometer (300MHz)

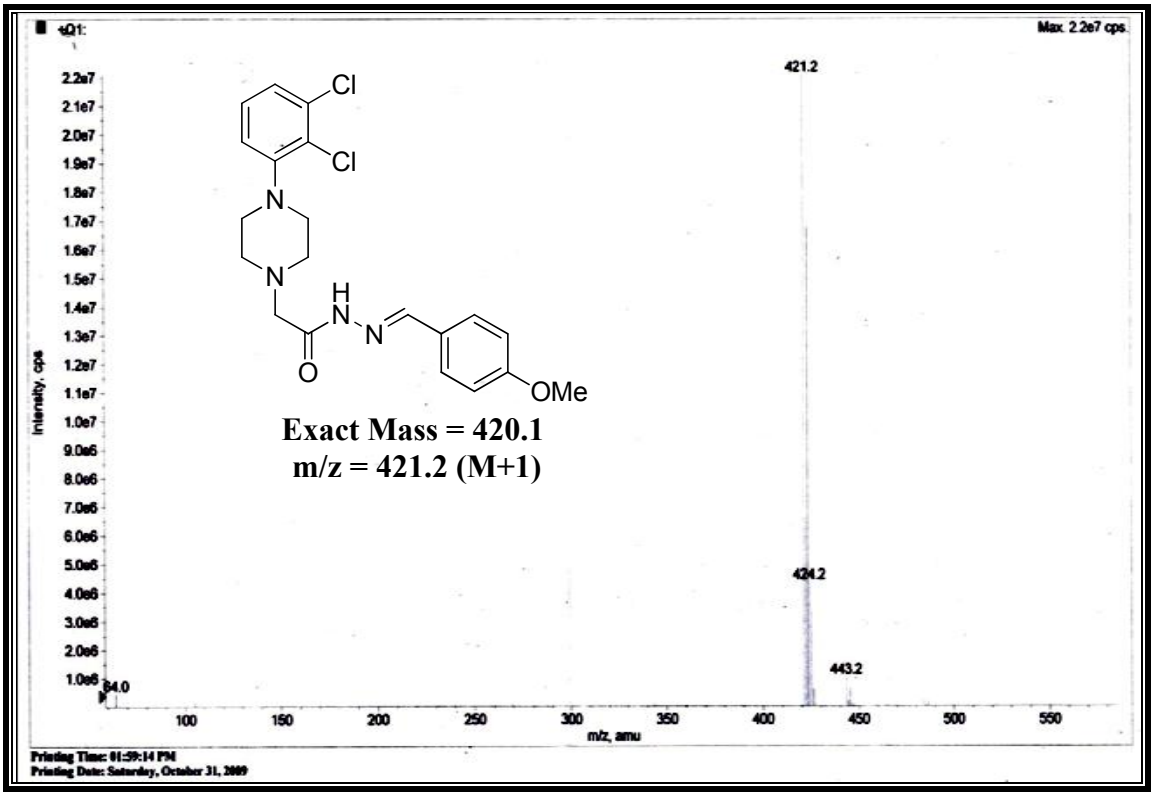
Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	2.79-2.82	4H	triplet	-C$\underline{H}_2$ (g,g')	-
2	3.12-3.14	4H	triplet	-C$\underline{H}_2$ (h,h')	-
3	3.28	2H	singlet	-C$\underline{H}_2$ (f)	-
4	3.84	3H	singlet	-OC$\underline{H}_3$ (a)	-
5	6.91-6.99	3H	multiplet	Ar-H (b,b',i)	-
6	7.14-7.21	2H	multiplet	Ar-H (j,k)	-
7	7.70-7.73	2H	doublet	Ar-H(c,c')	8.7
8	8.16	1H	singlet	-N=C$\underline{H}$ (d)	-
9	10.00	1H	singlet	-CO-N$\underline{H}$ (e)	-

EXPANDED ^{1}H-NMR SPECTRUM

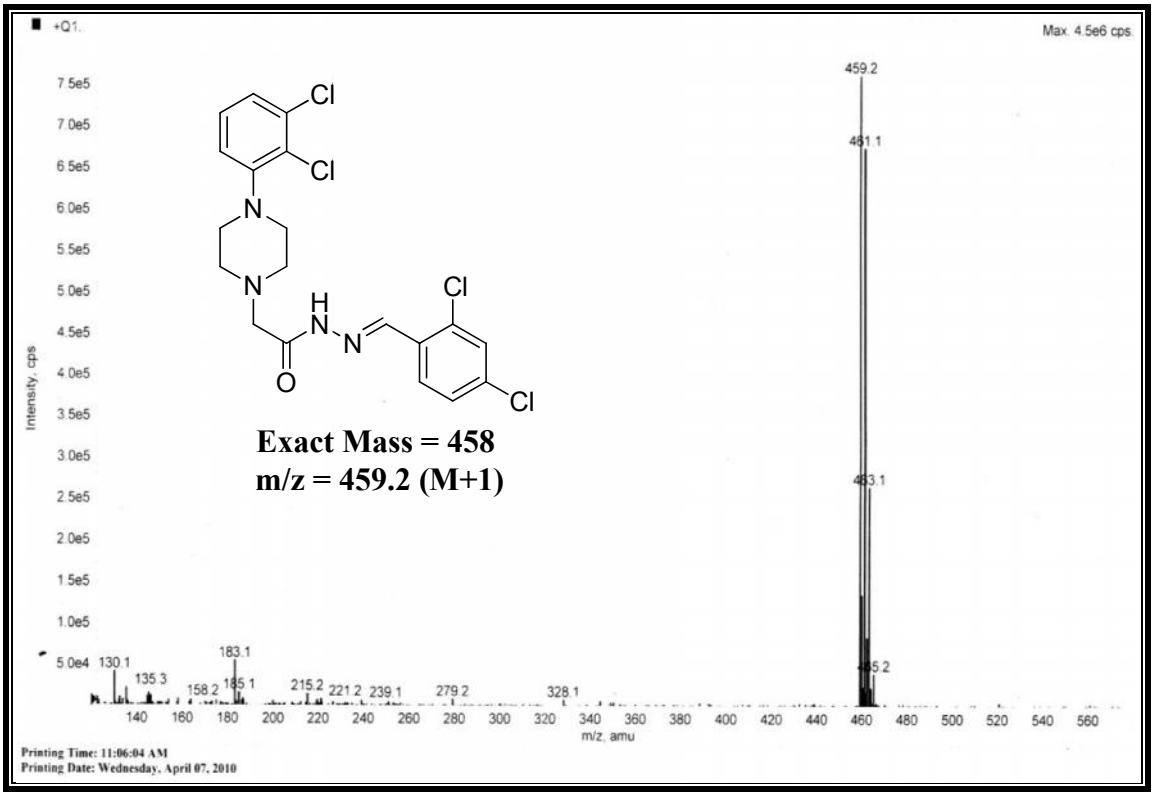




MASS SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*'-((4-METHOXYPHENYL)METHYLIDENE)ACETOHYDRAZIDE



MASS SPECTRUM OF *N*'-((2,4-DICHLOROPHENYL)METHYLIDENE)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE



EXPERIMENTAL

SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-ARYLMETHYLIDENE-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. 60 % Ethyl acetate in hexane was used as a mobile phase.

[A] SYNTHESIS OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

See, Chapter-2, Part-I, Section-I, Experimental [B], page no. 72.

[B] SYNTHESIS OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*'-((4-METHOXYPHENYL)METHYLIDENE)ACETOHYDRAZIDE

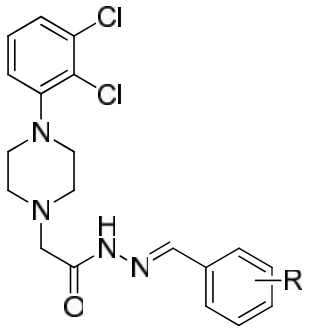
A mixture of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide (3.02 gm, 0.01 mol) and 4-methoxybenzaldehyde (1.36 gm, 0.01 mol) in ethanol (20 ml) in presence of catalytic amount of glacial acetic acid (2-3 drops) was refluxed for 4 hrs. The contents were cooled and poured in crused ice so solid precipitation was obtained, The product was filtered and dried. Isolated product was crystallized from ethanol. Yield: 85 %, M. P. 133-136 °C ($C_{20}H_{22}Cl_2N_4O_2$; Required: C, 57.01; H, 5.26; N, 13.30; Found: C, 56.84; H, 5.14; N, 13.18 %).

Similarly other *N*'-arylmethylidene-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide **(3a-j)** were prepared. The physical constants are recorded in **Table-3a,** Page no. 110.

[C] BIOLOGICAL SCREENING OF *N*'-ARYLMETHYLIDENE-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

Antimicrobial testing was carried out as described in Chapter-1**,** Section-I, Experimental [C], Page no. 37. The results obtained from antimicrobial testing are recorded in **Table-3b,** Page no. 111.

TABLE-3a: PHYSICAL CONSTANTS OF *N*'-ARYLMETHYLIDENE-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE



Sr. No.	Substitution R	Molecular Formula/ Molecular Weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
3a	H	$C_{19}H_{20}Cl_2N_4O$ 391.29	176-178	88	58.32 58.04	5.15 5.09	14.32 14.16
3b	4-OMe	$C_{20}H_{22}Cl_2N_4O_2$ 421.32	133-136	83	57.01 56.84	5.26 5.14	13.30 13.18
3c	4-F	$C_{19}H_{19}Cl_2FN_4O$ 409.28	191-194	94	55.76 55.49	4.68 4.56	13.69 13.54
3d	3-Cl	$C_{19}H_{19}Cl_3N_4O$ 425.74	171-173	81	53.60 53.41	4.50 4.38	13.16 13.11
3e	2,4-di Cl	$C_{19}H_{18}Cl_4N_4O$ 460.18	188-191	76	49.54 49.46	3.94 3.88	12.17 12.04
3f	4-$N(Me)_2$	$C_{21}H_{25}Cl_2N_5O$ 434.36	154-156	84	58.07 57.89	5.80 5.72	16.12 16.07
3g	2,5-di OMe	$C_{21}H_{24}Cl_2N_4O_3$ 451.35	178-181	79	55.88 55.79	5.36 5.32	12.41 12.36
3h	4-NO_2	$C_{19}H_{19}Cl_2N_5O_3$ 436.29	230 decompose	89	52.31 52.20	4.39 4.33	16.05 15.91
3i	4-OH	$C_{19}H_{20}Cl_2N_4O_2$ 407.29	170-171	86	56.03 55.84	4.95 4.82	13.76 13.64
3j	2-OH	$C_{19}H_{20}Cl_2N_4O_2$ 407.29	183-186	82	56.03 55.87	4.95 4.87	13.76 13.69

TABLE-3b: BIOLOGICAL SCREENING OF *N*'-ARYLMETHYLIDENE-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	3a	500	500	100	200	1000	500	500
2	3b	250	250	**62.5**	100	1000	1000	>1000
3	3c	250	500	250	**50**	500	500	500
4	3d	500	500	200	200	**250**	500	500
5	3e	250	500	200	200	1000	1000	1000
6	3f	**200**	200	500	250	**200**	1000	>1000
7	3g	500	500	200	100	**250**	1000	>1000
8	3h	500	100	250	250	1000	500	500
9	3i	**100**	100	200	250	500	250	250
10	3j	250	250	200	200	1000	200	200

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted Schiff bases **3f** (R= 4-$N(Me)_2$) & **3i** (R= 4-OH) against *S.aureus*, **3b** (R= 4-OMe) against *E-coli* and **3c** (R= 4-F) against *P.aeruginosa* possesses excellent activity compare to ampicillin. While **3c** (R= 4-F) & **3e** (R= 2,4-di Cl) against *S.aureus,* **3h** (R= 4-NO_2) & **3i** (R= 4-OH) against *S.pyogenus*, **3a** (R= -H) against *E-coli* and **3b** (R= 4-OMe) & **3g** (R= 2,5-di OMe) against *P.aeruginosa,* possess moderate activity as compare to ampicillin. The remaining schiff bases display moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted schiff bases **3d** (R= 3-Cl), **3f** (R= 4-$N(Me)_2$) & **3g** (R= 2,5-di OMe) show highly promising activity against *C.albicans* compare to greseofulvin. While **3i** (R = 4-OH) & **3j** (R = 2-OH) possess good activity against *A.niger* and *A.clavatus* compare to standard drug. The remaining compounds exhibit moderate to poor activity.

SECTION-II

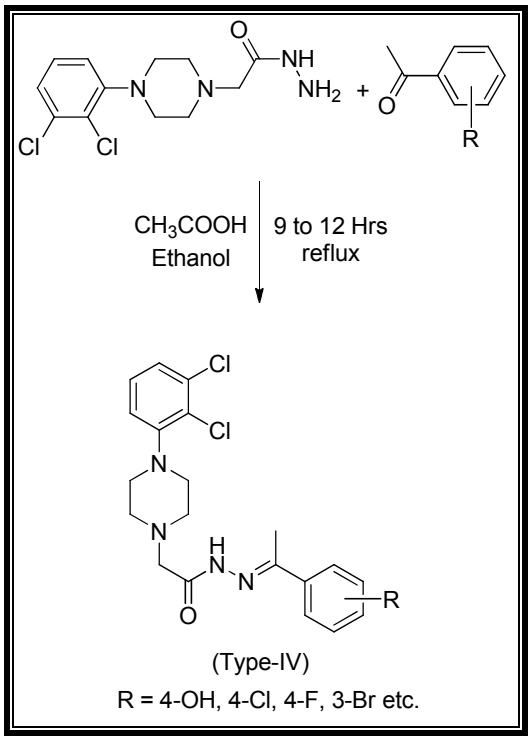
SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-(1-ARYLETHYLIDENE)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

Schiff base play a vital role owing of their wide range of biological activity and with an aim to synthesised better molecule, it was considered worthwhile to synthesize some azomethine derivatives of Type-(IV) by the condensation of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide with different aromatic acetophenones in order to study their biodynamic behavior.

The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

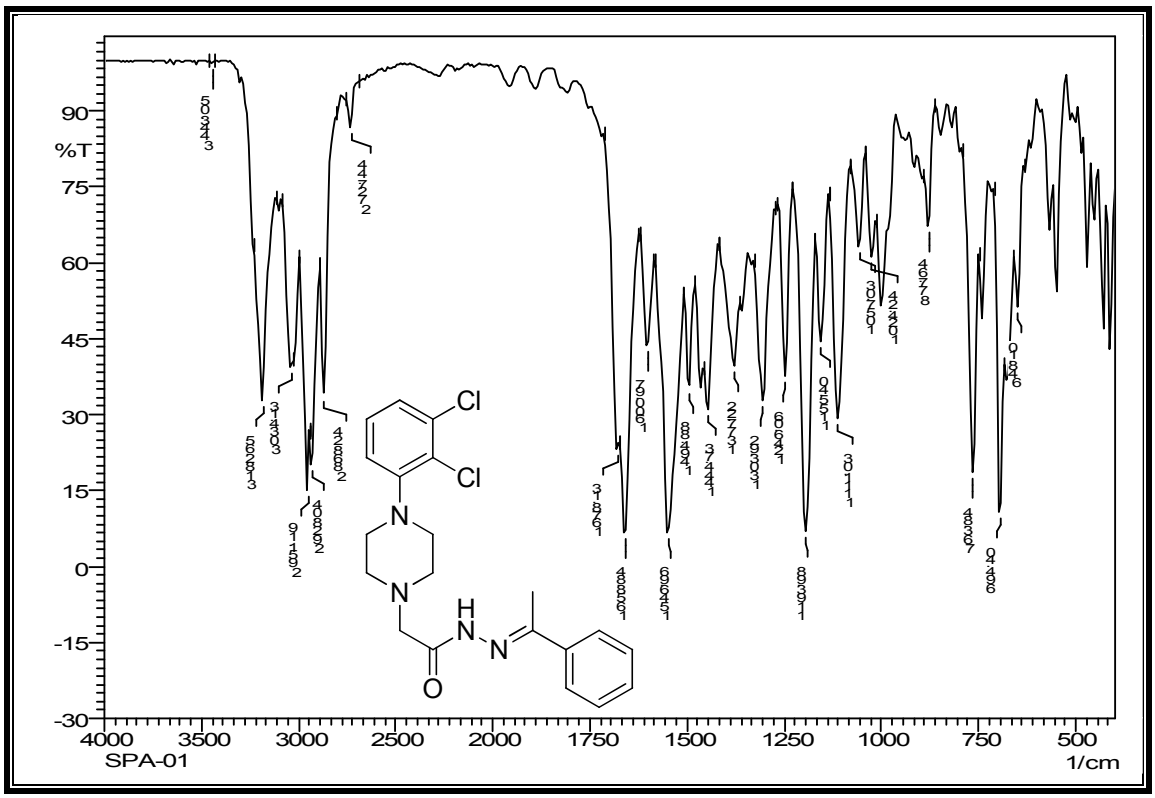
REACTION SCHEME



(Type-IV)

R = 4-OH, 4-Cl, 4-F, 3-Br etc.

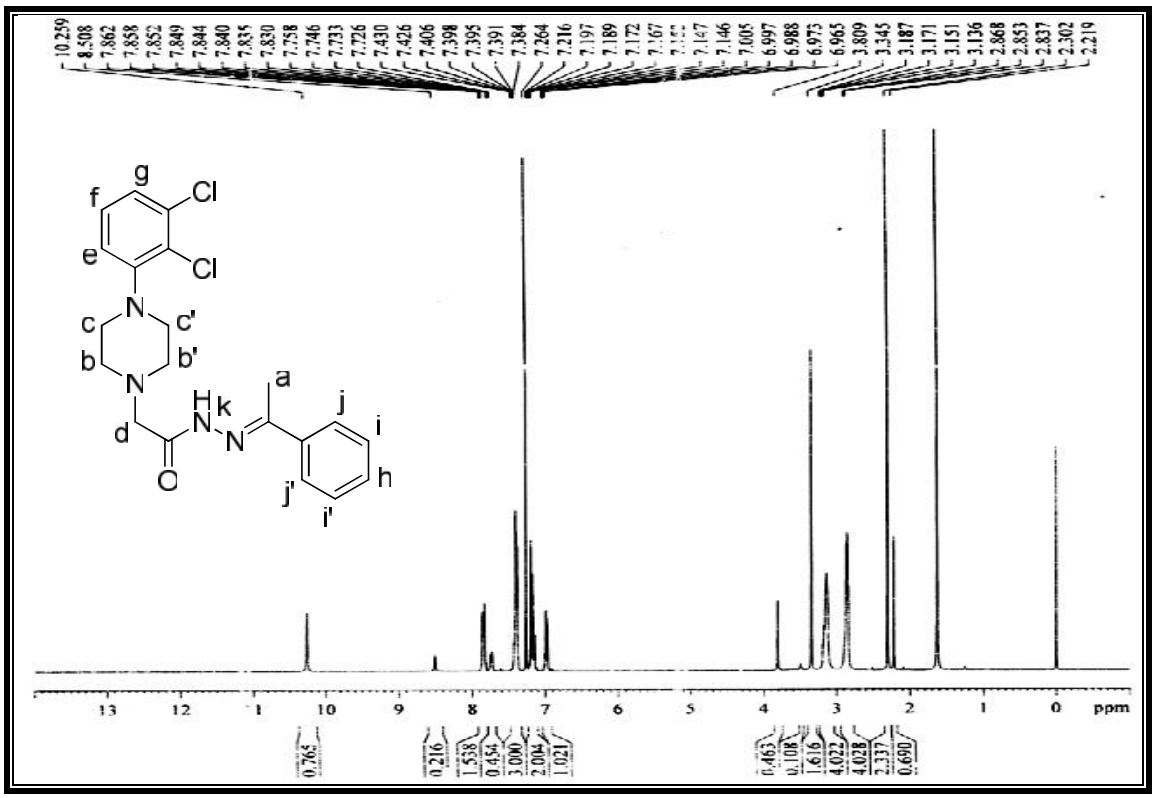
IR SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*'-(1-PHENYLETHYLIDENE)ACETOHYDRAZIDE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2951	2975-2920	112
	C-H str. (sym.)	2868	2880-2860	”
	C-H def. (asym.)	1444	1470-1435	”
	C-H def. (sym.)	1377	1395-1370	”
Aromatic	C-H str.	3034	3100-3000	”
	C=C str.	1494	1585-1480	”
	C-H i.p. def.	1111	1125-1090	”
	C-H o.o.p. def.	877	860-810	”
Azomethine	N=C str.	1600	1650-1580	”
	C-N str.	1246	1350-1200	”
Carbonyl	C=O	1658	1700-1650	”
Halide	C-Cl	763	850-650	”

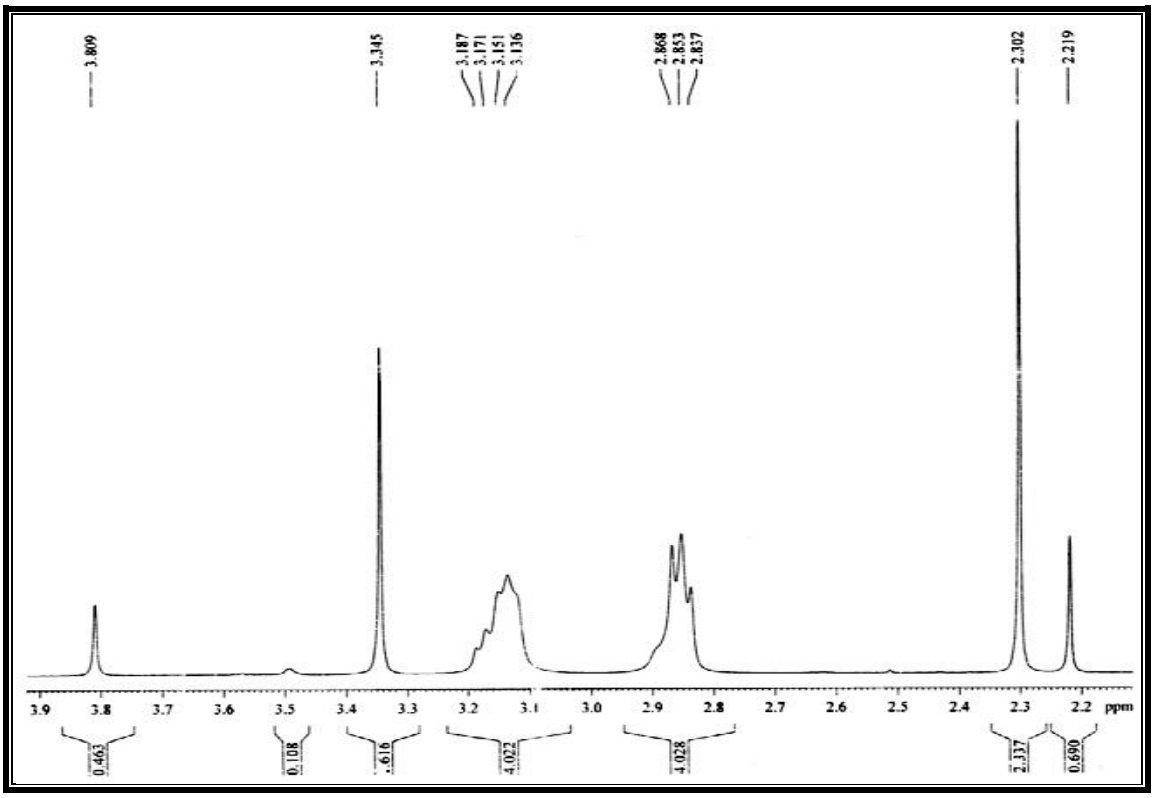
^{1}H-NMR SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N'*-(1-PHENYLETHYLIDENE)ACETOHYDRAZIDE

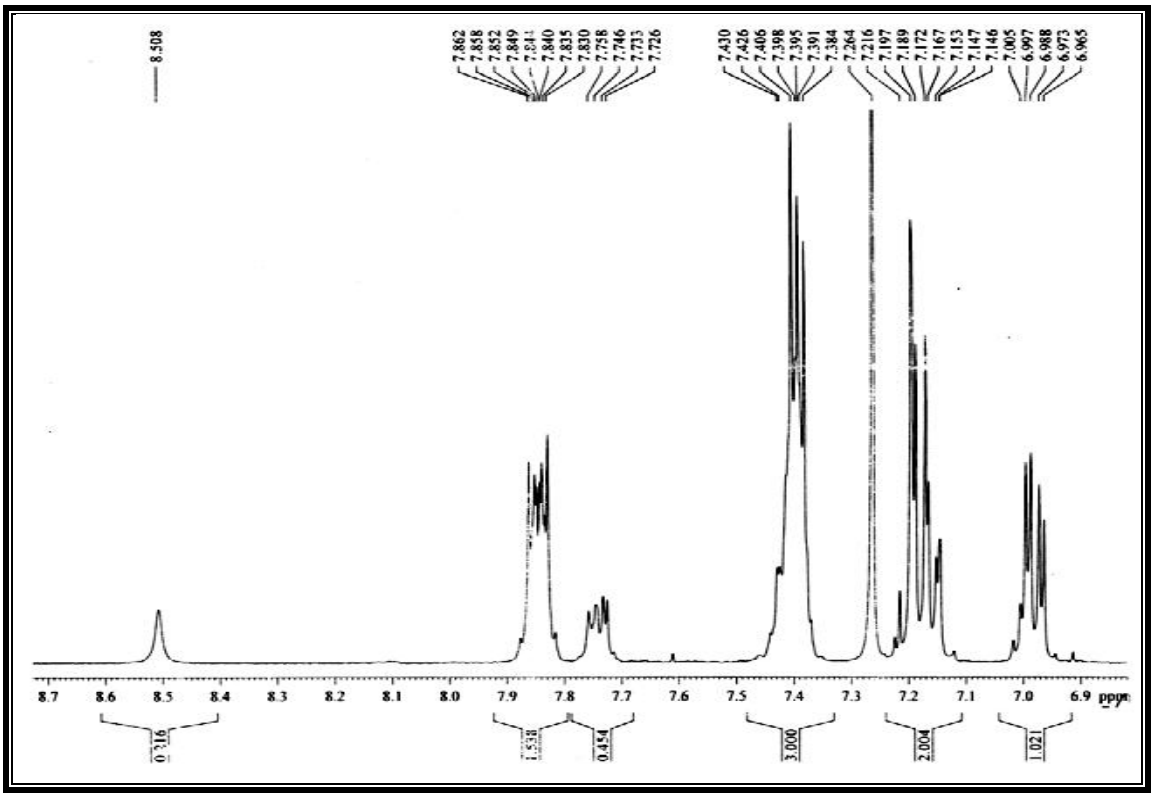


Internal Standard: TMS; Solvent: $CDCl_3$ Instrument: BRUKER Spectrometer (300MHz)

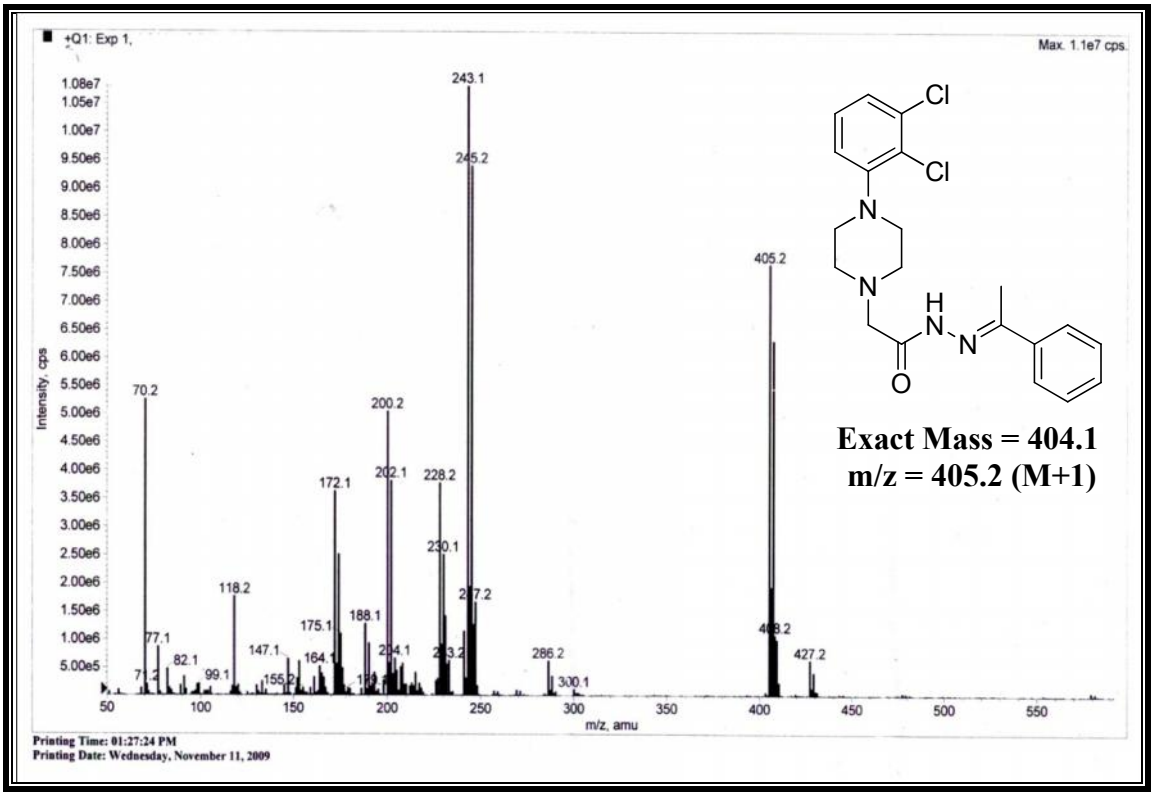
Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	2.30	3H	singlet	-C<u>H</u>$_3$(a)	-
2	2.83-2.86	4H	triplet	-C<u>H</u>$_2$ (b,b')	-
3	3.13-3.17	4H	triplet	-C<u>H</u>$_2$ (c,c')	-
4	3.34	2H	singlet	-C<u>H</u>$_2$ (d)	-
5	6.96-7.00	1H	multiplet	Ar-H (e)	-
6	7.14-7.21	2H	multiplet	Ar-H (f,g)	-
7	7.38-7.43	3H	multiplet	Ar-H(h,i,i')	-
8	7.83-7.86	2H	multiplet	Ar-H(j,j')	-
9	10.25	1H	singlet	-CO-N<u>H</u> (k)	-

EXPANDED ^{1}H-NMR SPECTRUM

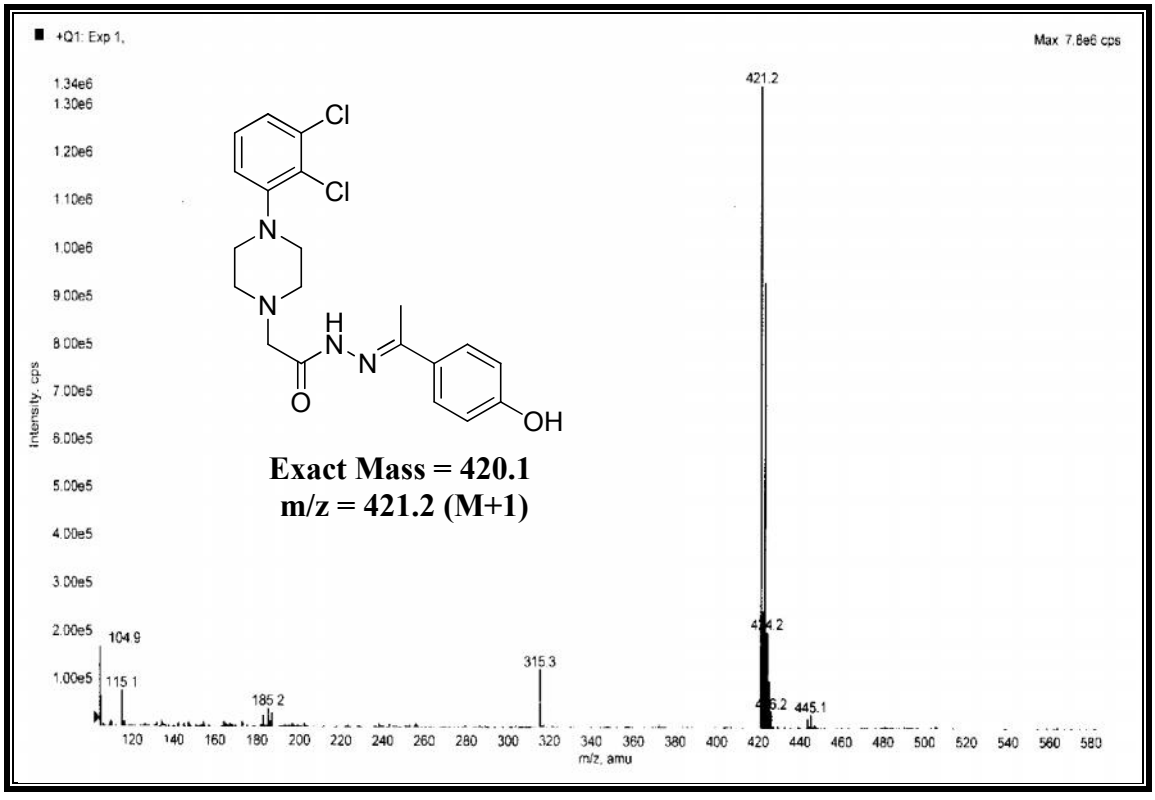




MASS SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N'*-(1-PHENYLETHYLIDENE)ACETOHYDRAZIDE



MASS SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N'*-(1-(4-HYDROXYPHENYL)ETHYLIDENE)ACETOHYDRAZIDE



EXPERIMENTAL

SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-(1-ARYLETHYLIDENE)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. 50 % Ethyl acetate in hexane was used as a mobile phase.

[A] SYNTHESIS OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

See, Chapter-2, Part-I, Section-I, Experimental [B], page no. 72.

[B] SYNTHESIS OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N'*-(1-PHENYLETHYLIDENE)ACETOHYDRAZIDE

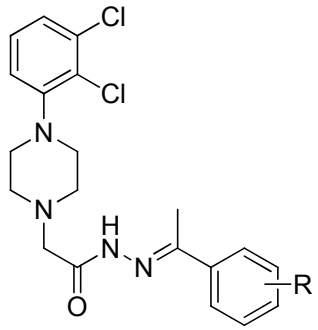
A mixture of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide (3.02 gm, 0.01 mol) and acetophenone (1.20 gm, 0.01 mol) in ethanol (20 ml) in presence of catalytic amount of glacial acetic acid was refluxed for 10 hrs. The contents were cooled and poured in crused ice so solid precipitation was obtained, The product was filtered and dried. Isolated product was crystallized from ethanol. Yield: 85 %, M. P. 175-178 °C, ($C_{20}H_{22}Cl_2N_4O$; Required: C, 59.27; H, 5.47; N, 13.82; Found: C, 59.09; H, 5.41; N, 13.73 %).

Similarly, other *N*'-(1-arylethylidene)-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide **(4a-j)** were prepared. The physical constants are recorded in **Table-4a**, Page no. 119.

[C] BIOLOGICAL SCREENING OF *N*'-(1-ARYLETHYLIDENE)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

Antimicrobial testing was carried out as described in Chapter-1**,** Section-I, Experimental [C], Page no. 37. The results obtained from antimicrobial testing are recorded in **Table-4b,** Page no. 120.

TABLE-4a: PHYSICAL CONSTANTS OF *N*'-(1-ARYLETHYLIDENE)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE



Sr. No.	Substitution R	Molecular Formula/ Molecular Weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
4a	H	$C_{20}H_{22}Cl_2N_4O$ 405.32	175-178	85	59.27 59.09	5.47 5.41	13.82 13.73
4b	4-Me	$C_{21}H_{24}Cl_2N_4O$ 419.35	151-153	81	60.15 60.01	5.77 5.71	13.36 13.28
4c	4-F	$C_{20}H_{21}Cl_2FN_4O$ 423.31	185-188	91	56.75 56.54	5.00 4.94	13.24 13.18
4d	4-Cl	$C_{20}H_{21}Cl_3N_4O$ 439.77	176-178	83	54.62 54.48	4.81 4.77	12.74 12.59
4e	2,4-di Cl	$C_{20}H_{20}Cl_4N_4O$ 474.21	163-165	73	50.66 50.49	4.25 4.19	11.81 11.73
4f	4-Br	$C_{20}H_{21}BrCl_2N_4O$ 484.22	181-182	88	49.61 49.53	4.37 4.33	11.57 11.45
4g	3-Br	$C_{20}H_{21}BrCl_2N_4O$ 484.22	198-201	83	49.61 49.43	4.37 4.29	11.57 11.46
4h	4-OH	$C_{20}H_{22}Cl_2N_4O_2$ 421.32	188-191	79	57.01 56.84	5.26 5.14	13.30 13.21
4i	4-NH_2	$C_{20}H_{23}Cl_2N_5O$ 420.34	176-179	75	57.15 56.91	5.52 5.43	16.66 16.52
4j	2-NO_2	$C_{20}H_{21}Cl_2N_5O_3$ 450.32	211-213	80	53.34 53.11	4.70 4.66	15.55 15.44

TABLE-4b: BIOLOGICAL SCREENING OF *N*'-(1-ARYLETHYLIDENE)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	4a	250	200	500	500	500	500	500
2	4b	**200**	200	100	250	**250**	500	500
3	4c	500	100	250	250	500	1000	1000
4	4d	500	500	200	250	500	1000	1000
5	4e	250	500	250	500	**250**	500	500
6	4f	500	500	200	250	1000	500	500
7	4g	500	500	100	500	1000	500	500
8	4h	250	250	500	250	1000	200	200
9	4i	250	250	**62.5**	100	>1000	>1000	>1000
10	4j	500	250	500	500	**200**	>1000	>1000

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted Schiff base **4b** (R= 4-Me) against *S.aureus* and **4i** (R= 4-NH_2) against *E-coli* exhibit outstanding activity compare to ampicillin. While **4e** (R= 2,4-di Cl) & **4i** (R= 4-NH_2) against *S.aureus,* **4c** (R= 4-F) against *S.pyogenus*, **4b** (R= 4-Me) & **4g** (R= 3-Br) against *E-coli* and **4i** (R= 4-NH_2) against *P.aeruginosa,* possess moderate activity as compare to ampicillin. The remaining schiff bases show moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data showed that substituted schiff bases **4b** (R= 4-Me), **4e** (R= 2,4-di Cl) & **4j** (R= 2-NO_2) display highly promising activity against *C.albicans* as compare to greseofulvin. While **4h** (R = 4-OH) possess good activity against *A.niger* and *A.clavatus* compare to standard drug. The remaining compounds exhibit moderate to poor activity.

SECTION-III

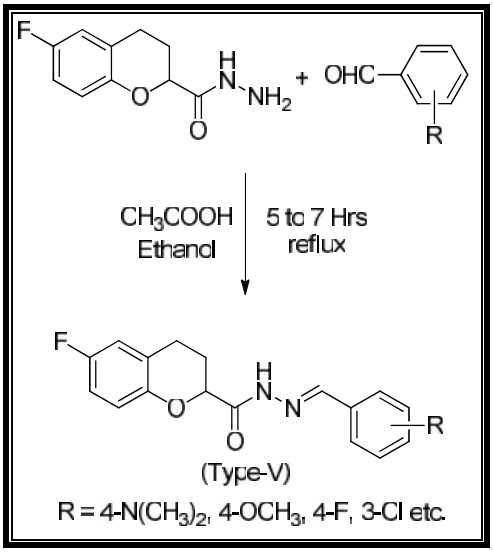
SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-ARYLMETHYLIDENE-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

Schiff's base derivatives represent one of the modest classes of biological active agent which have been deeply studies during search on new potential drugs, these have been reported to be active as antimicrobial, antitubercular, anthelmintics etc. In view of these findings, it appeared of interest to synthesize Schiff's base derivatives of Type-(V) by the condensation of 6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide with different aromatic aldehydes.

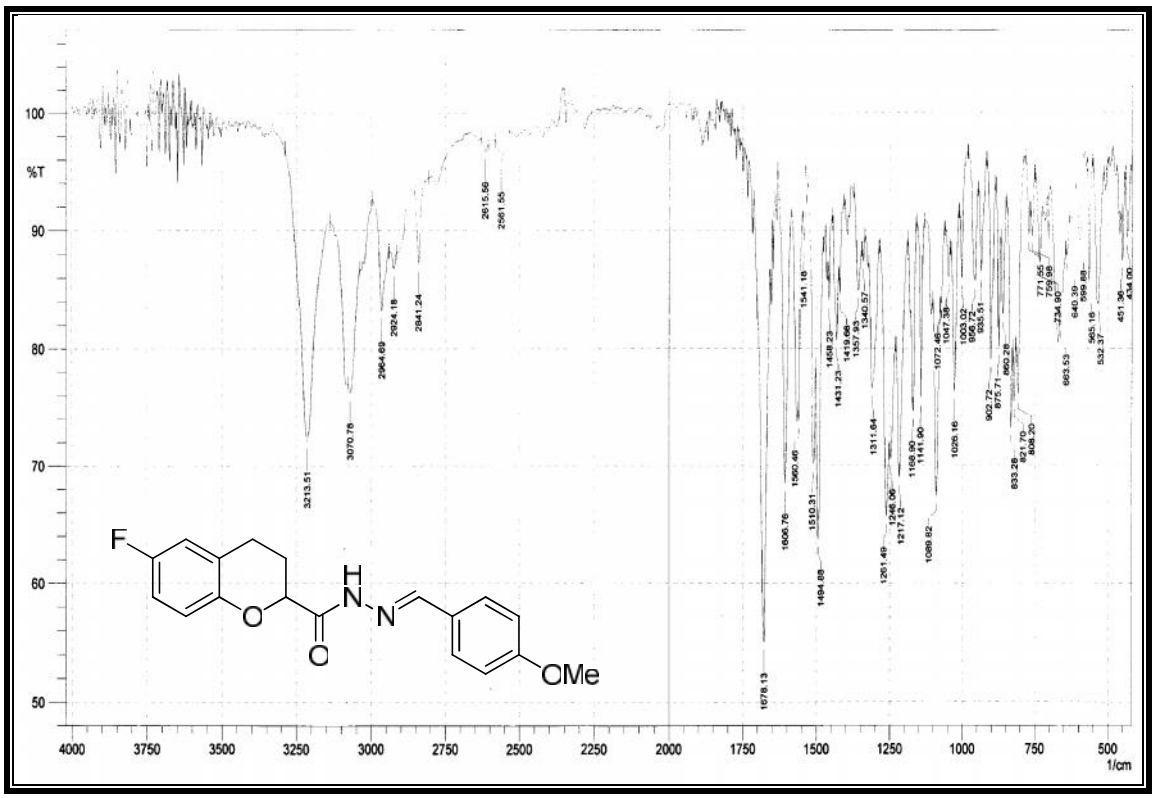
The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

REACTION SCHEME



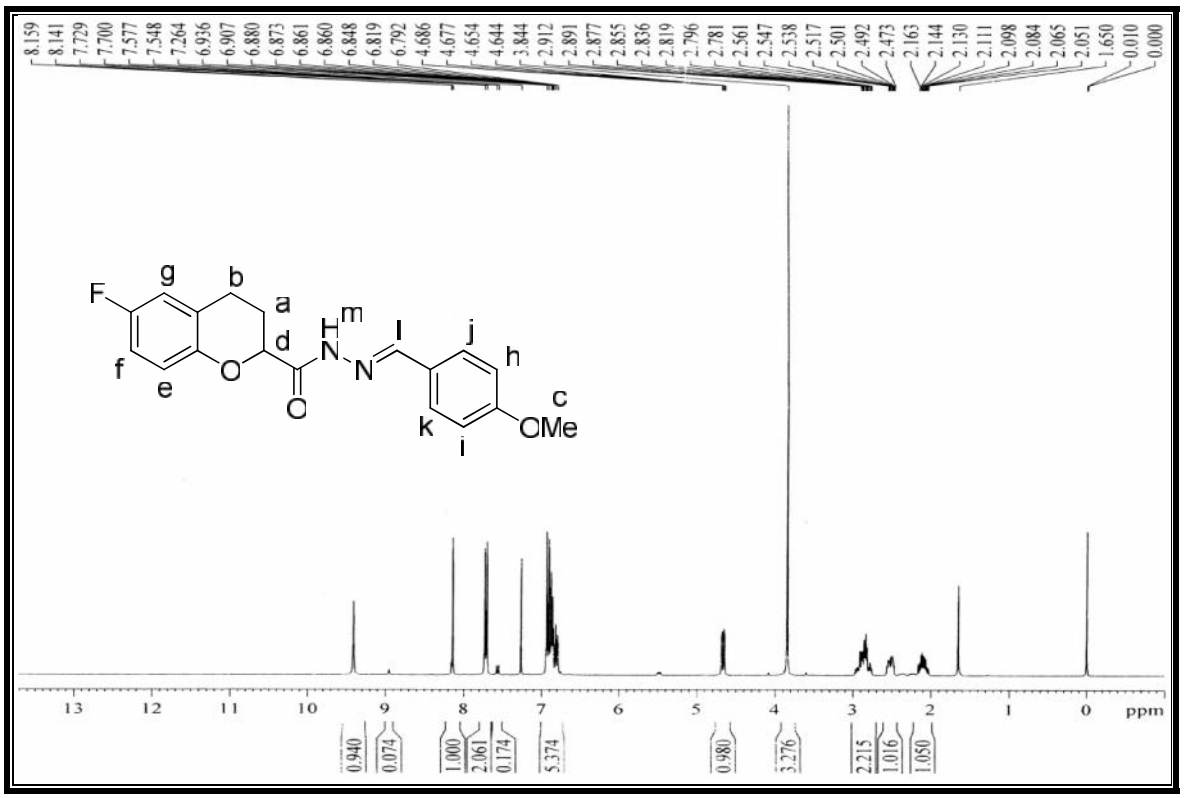
IR SPECTRUM OF 6-FLUORO-*N*'-((4-METHOXYPHENYL)METHYLIDENE)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2964	2975-2920	112
	C-H str. (sym.)	2841	2880-2860	”
	C-H def. (asym.)	1431	1470-1435	”
	C-H def. (sym.)	1357	1395-1370	”
Aromatic	C-H str.	3070	3100-3000	”
	C=C	1510	1585-1480	”
	C-H i.p. def.	1089	1125-1090	”
	C-H o.o.p. def.	833	860-810	”
Azomethine	N=C str.	1606	1650-1580	”
	C-N str.	1311	1350-1200	”
Carbonyl	C=O	1678	1700-1650	”
Ether	C-O-C	1261	1275-1200	”
Amide	-NH str.	3213	3200-3400	”

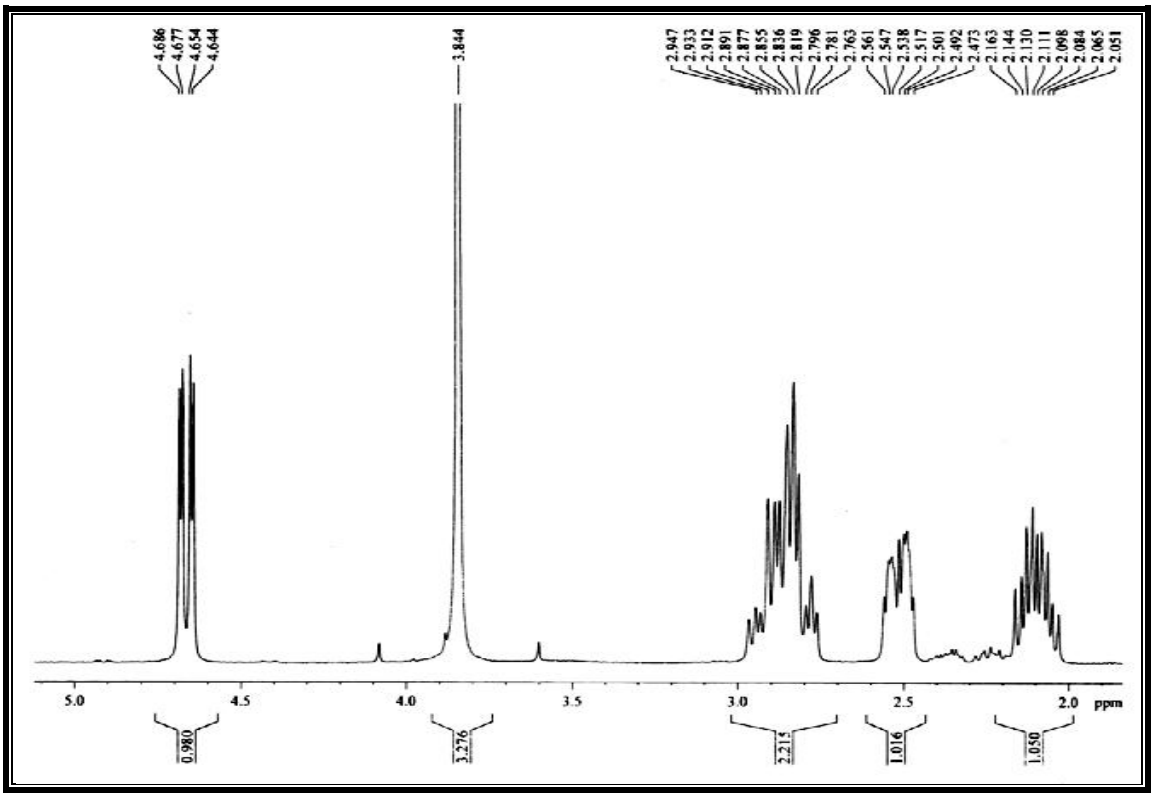
^{1}H-NMR SPECTRUM OF 6-FLUORO-*N*'-((4-METHOXYPHENYL)METHYLIDENE)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

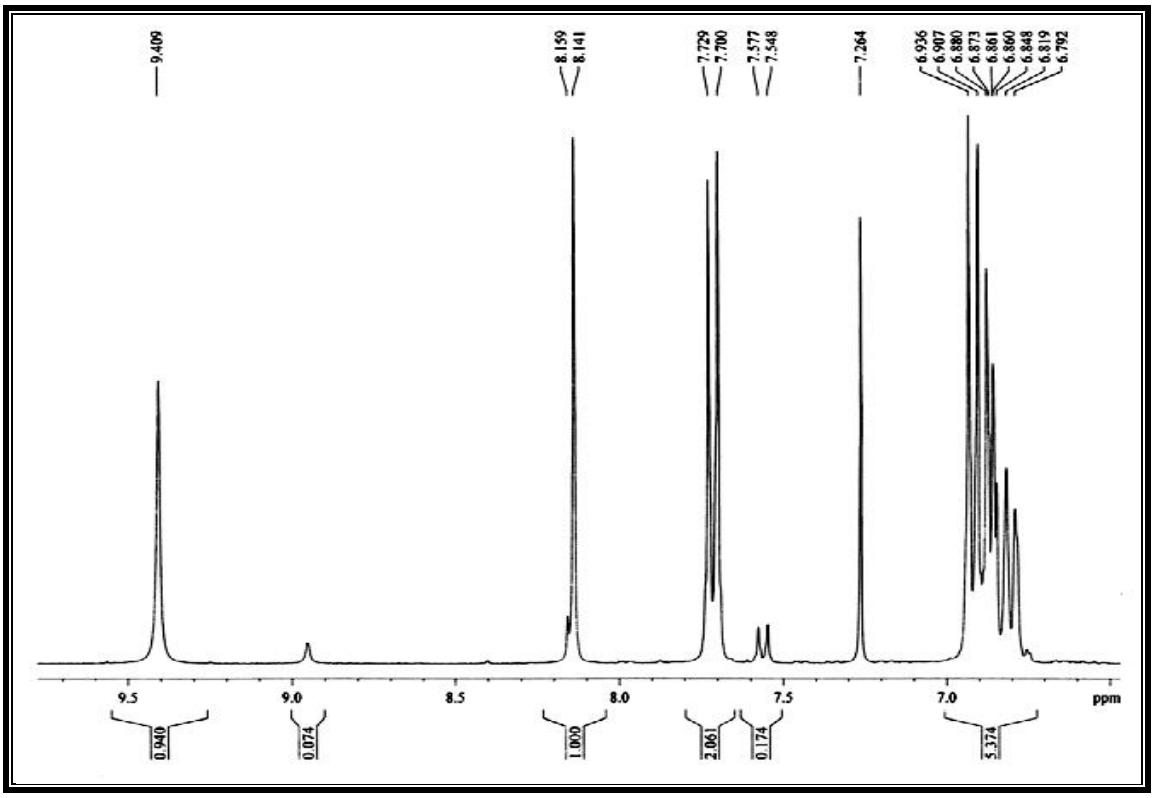


Internal Standard: TMS; Solvent: $CDCl_3$ Instrument: BRUKER Spectrometer (300MHz)

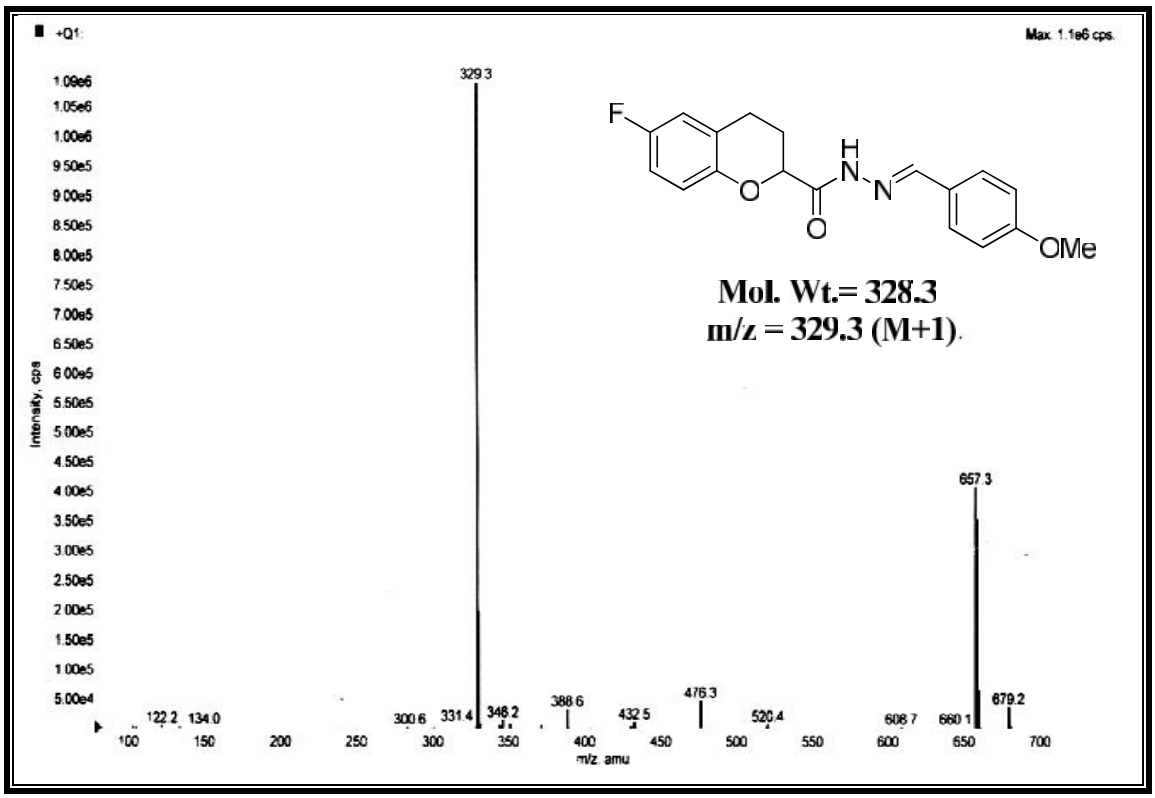
Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	2.05-2.16	1H	multiplet	-C<u>H</u>$_2$ (a)	-
2	2.47-2.56	1H	multiplet	-C<u>H</u>$_2$ (a)	-
3	2.76-2.94	2H	multiplet	-C<u>H</u>$_2$ (b)	-
4	3.84	3H	singlet	-OC<u>H</u>$_3$ (c)	-
5	4.64-4.68	1H	double doublet	-C<u>H</u> (d)	3.0 & 9.9
6	6.79-6.93	5H	multiplet	Ar-H (e to i)	-
7	7.70-7.72	2H	doublet	Ar-H(j,k)	8.7
8	8.14	1H	singlet	-N=C<u>H</u> (l)	-
9	9.40	1H	singlet	-CO-N<u>H</u> (m)	-

EXPANDED ^{1}H-NMR SPECTRUM

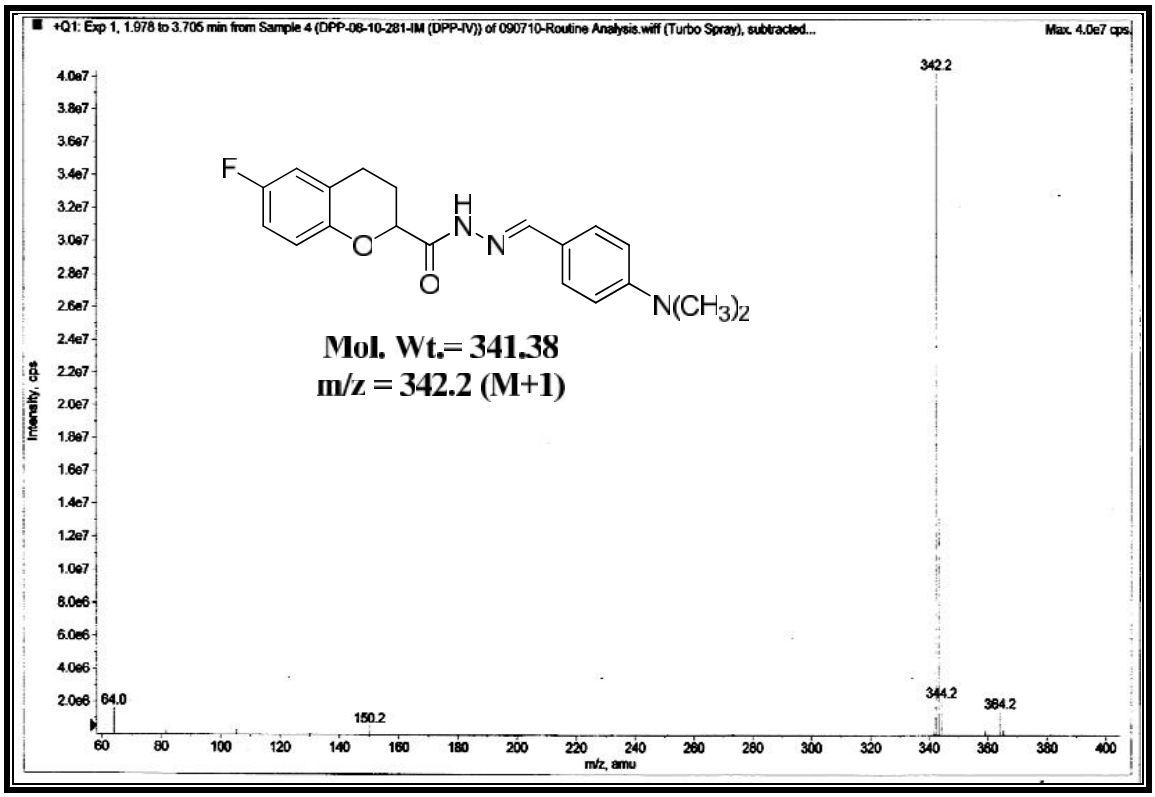




MASS SPECTRUM OF 6-FLUORO-*N*'-((4-METHOXYPHENYL)METHYLIDENE)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



MASS SPECTRUM OF *N*'-((4-(DIMETHYLAMINO)PHENYL)METHYLIDENE)-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



EXPERIMENTAL

SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-ARYLMETHYLIDENE-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. 60 % Ethyl acetate in hexane was used as a mobile phase.

[A] SYNTHESIS OF 6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

See, Chapter-2, Part-II, Section-I, Experimental [B], page no. 93.

[B] SYNTHESIS OF 6-FLUORO-*N*'-((4-METHOXYPHENYL)METHYLIDENE)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

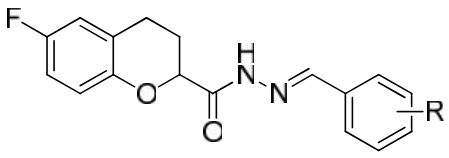
A mixture of 6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide (2.10 gm, 0.01 mol) and 4-methoxybenzaldehyde (1.36 gm, 0.01 mol) dissolved in ethanol (20 ml) in presence of catalytic amount of glacial acetic acid was refluxed for 6 hrs. The contents were cooled and poured in crused ice so solid precipitation was obtained, The product was filtered and dried. Isolated product was crystallized from ethanol. Yield: 85 %, M. P. 164-167 °C, ($C_{18}H_{17}FN_2O_3$; Required: C, 65.84; H, 5.22; N, 8.53; Found: C, 65.56; H, 5.10; N, 8.41 %).

Similarly, other *N*'-arylmethylidene-6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide were **(5a-j)** prepared. The physical constants are recorded in **Table-5b,** Page no. 128.

[C] BIOLOGICAL SCREENING OF *N*'-ARYLMETHYLIDENE-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

Antimicrobial testing was carried out as described in Chapter-1**,** Section-I, Experimental Section-[C], Page no. 37. The results obtained from antimicrobial testing are recorded in **Table-5b,** Page no. 129.

TABLE 5a: PHYSICAL CONSTANTS OF *N*'-ARYLMETHYLIDENE-6-FLUORO--3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



Sr. No.	Substitution R	Molecular Formula/ Molecular Weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
5a	H	$C_{17}H_{15}FN_2O_2$ 298.31	158-160	79	68.45 68.29	5.07 5.01	9.39 9.23
5b	4-OMe	$C_{18}H_{17}FN_2O_3$ 328.34	164-167	85	65.84 65.56	5.22 5.10	8.53 8.41
5c	4-F	$C_{17}H_{14}F_2N_2O_2$ 316.30	173-176	81	64.55 64.22	4.46 4.39	8.86 8.72
5d	3-Cl	$C_{17}H_{14}ClFN_2O_2$ 332.76	151-153	86	61.36 61.08	4.24 4.19	8.42 8.27
5e	2,4-di Cl	$C_{17}H_{13}Cl_2FN_2O_2$ 367.20	182-183	77	55.60 55.43	3.57 3.51	7.63 7.51
5f	4-N(Me)$_2$	$C_{19}H_{20}FN_3O_2$ 341.38	166-169	80	66.85 66.62	5.91 5.85	12.31 12.24
5g	4-OH,3-OMe	$C_{18}H_{17}FN_2O_4$ 344.34	171-173	79	62.79 62.55	4.98 4.90	8.14 8.03
5h	4-NO2	$C_{17}H_{14}FN_3O_4$ 343.31	182-185	75	59.47 59.29	4.11 4.03	12.24 12.07
5i	4-OH	$C_{17}H_{15}FN_2O_3$ 314.31	165-167	87	64.96 64.80	4.81 4.73	8.91 8.82
5j	2-OH	$C_{17}H_{15}FN_2O_3$ 314.31	177-180	82	64.96 64.73	4.81 4.68	8.91 8.78

TABLE 5b: BIOLOGICAL SCREENING OF *N*'-ARYLMETHYLIDENE-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	5a	200	100	**62.5**	100	500	200	200
2	5b	200	200	200	**50**	1000	1000	1000
3	5c	**100**	500	100	250	500	>1000	>1000
4	5d	250	500	200	500	**250**	>1000	>1000
5	5e	500	250	200	250	1000	200	200
6	5f	500	500	100	100	**200**	>1000	>1000
7	5g	500	500	200	250	1000	>1000	>1000
8	5h	500	250	500	500	500	1000	1000
9	5i	**100**	100	500	500	500	1000	1000
10	5j	250	200	500	500	**250**	>1000	>1000

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted Schiff bases **5c** (R= 4-F) & **5i** (R = 4-OH) against *S.aureus,* **5a** (R= -H) against *E-coli* and **5b** (R= 4-OMe) against *P.aeruginosa* possess excellent activity compared to ampicillin. While **5a** (R= -H) & **5b** (R= 4-OMe) against *S.aureus,* **5a** (R= -H) & **5i** (R= 4-OH) against *S.pyogenus*, **5c** (R= 4-F) & **5f** (R= 4-$N(Me)_2$) against *E-coli* and **5a** (R= -H) & **5f** (R= 4-$N(Me)_2$) against *P.aeruginosa,* display moderate activity as compared to ampicillin. The remaining schiff bases demonstrate poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data showed that substituted schiff bases **5d** (R= 3-Cl), **5f** (R= 4-$N(Me)_2$) & **5j** (R= 2-OH) show very good activity against *C.albicans* as compared to greseofulvin. While **5a** (R= -H) & **5e** (R= 2,4-di Cl) exhibit good activity against *A.niger* and *A.clavatus* compare to standard drug. The remaining compounds display poor activity against all three bacterial species.

SECTION-IV

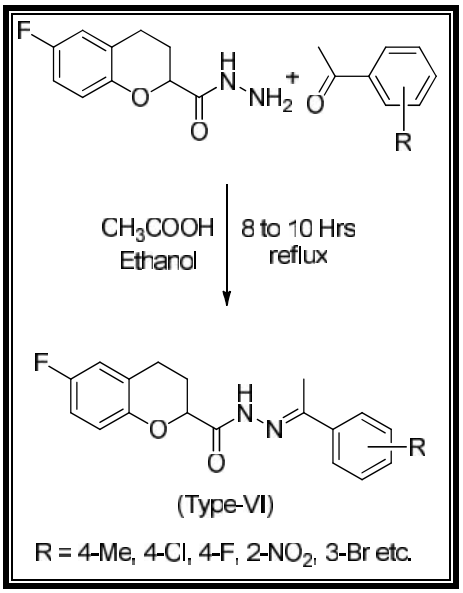
SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-(1-ARYLETHYLIDENE)-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRZIDE

Synthesis of schiff base derivatives has attracted considerable attention in view of therapeutic applications. Looking to this, schiff base derivative of Type-(VI) have been synthesised by the condensation of 6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide with different substituted aromatic acetophenones.

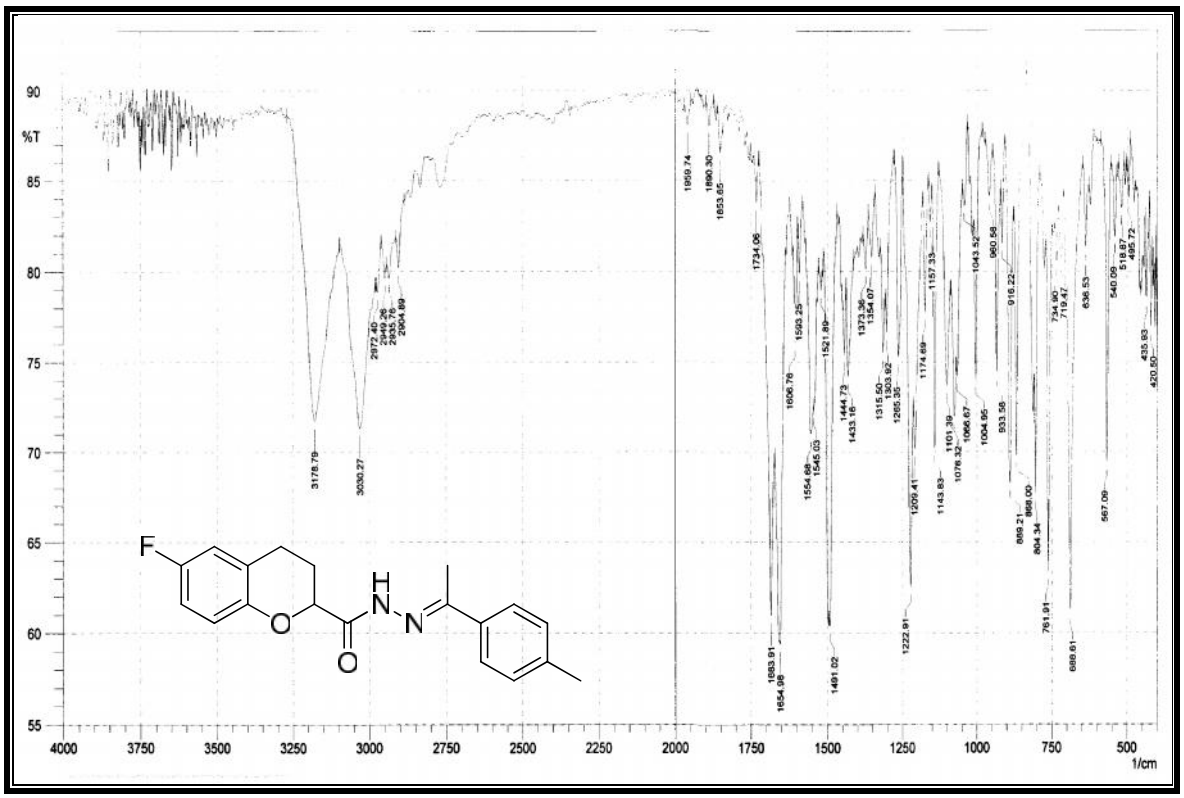
The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

REACTION SCHEME



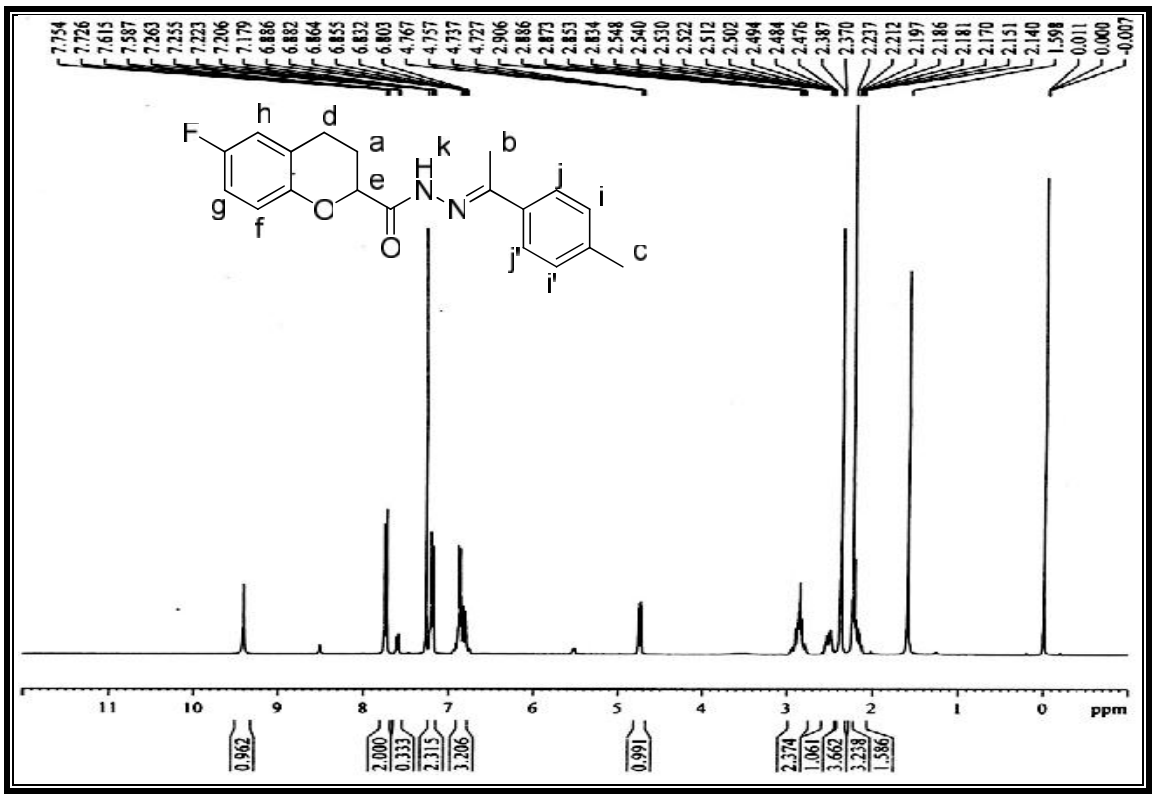
IR SPECTRUM OF 6-FLUORO-*N*'-(1-(4-METHYLPHENYL)ETHYLIDENE)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2935	2975-2920	112
	C-H str. (sym.)	2850	2880-2860	”
	C-H def. (asym.)	1444	1470-1435	”
	C-H def. (sym.)	1373	1395-1370	”
Aromatic	C-H str.	3030	3100-3000	”
	C=C	1554	1585-1480	”
	C-H i.p. def.	1101	1125-1090	”
	C-H o.o.p. def.	868	860-810	”
Azomethine	N=C str.	1654	1650-1580	”
	C-N str.	1265	1350-1200	”
Carbonyl	C=O	1683	1700-1650	”
Amide	-NH str.	3178	3200-3400	”

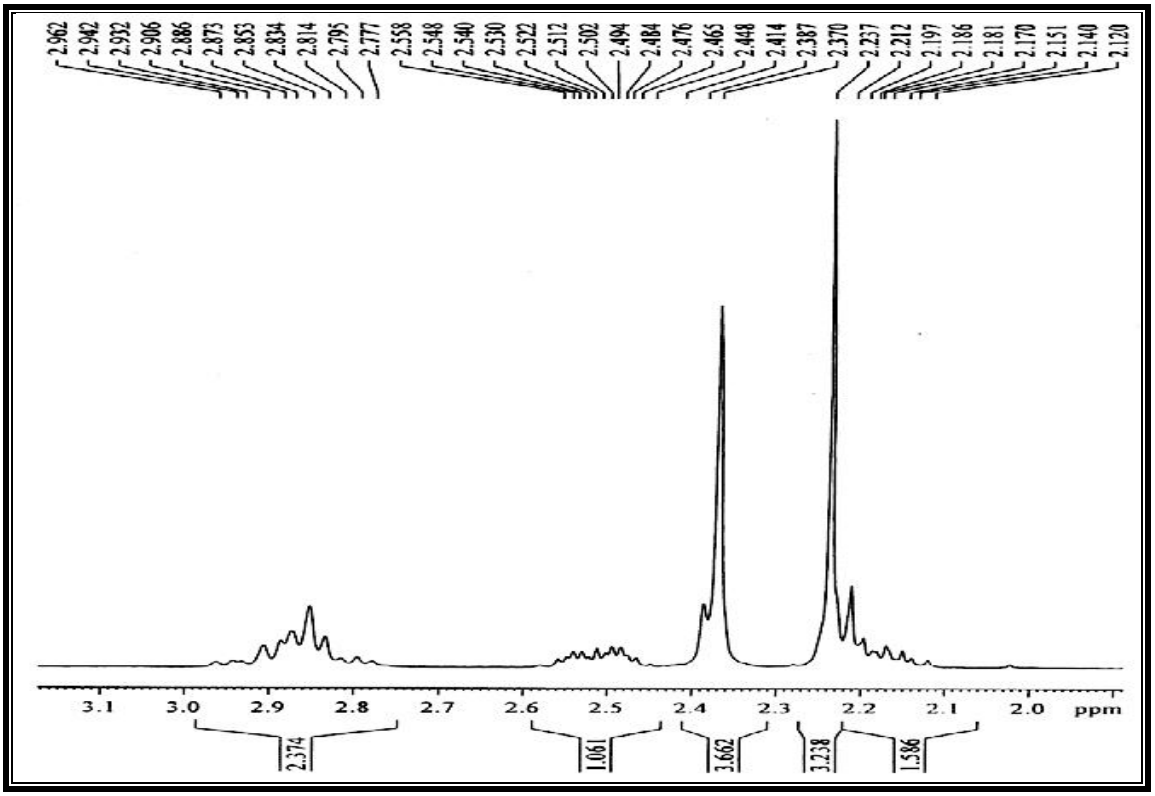
^{1}H-NMR SPECTRUM OF 6-FLUORO-*N*'-(1-(4-METHYLPHENYL)ETHYLIDENE)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

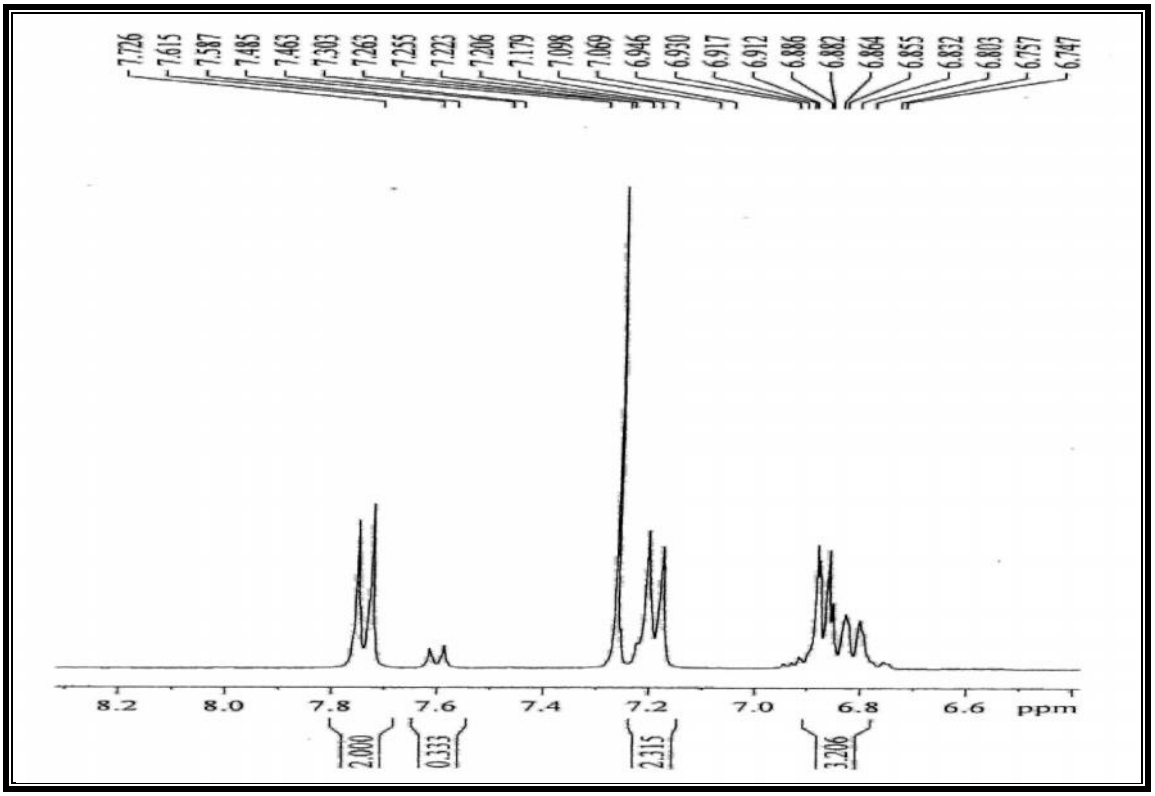


Internal Standard: TMS; Solvent: $CDCl_3$ Instrument: BRUKER Spectrometer (300MHz)

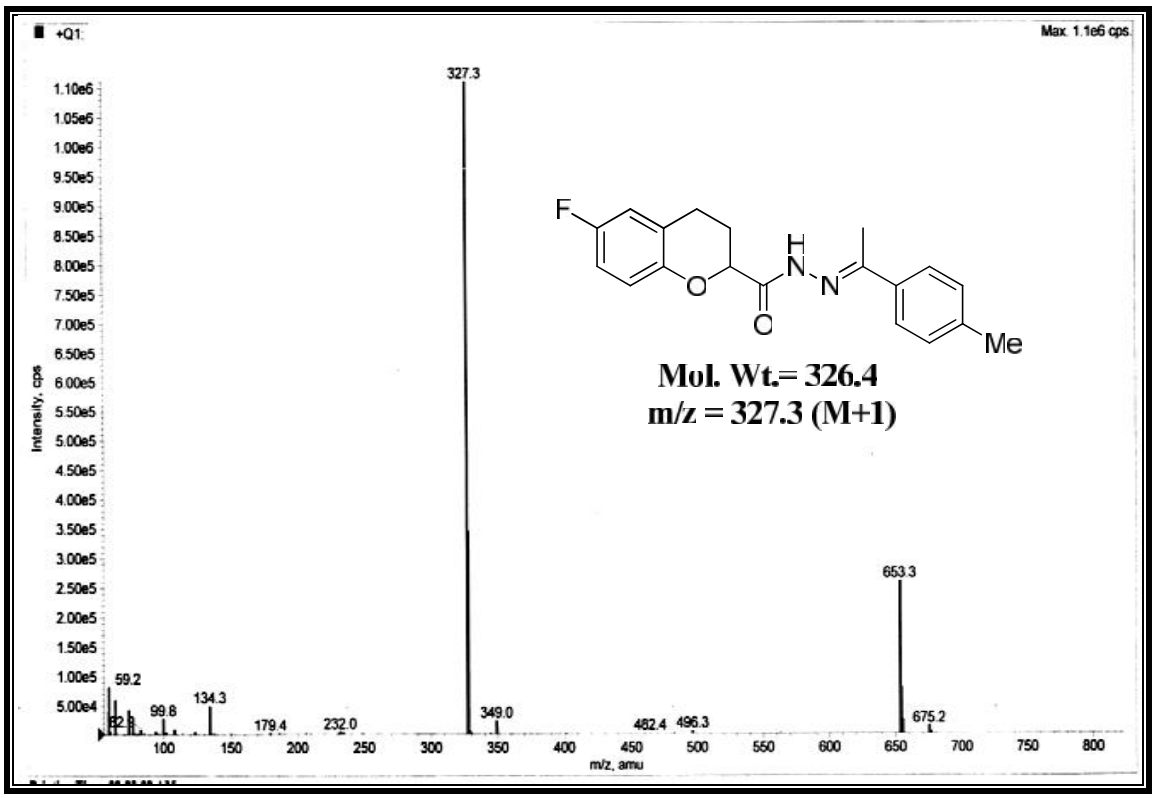
Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	2.12-2.21	1H	multiplet	-C<u>H</u>$_2$ (a)	-
2	2.23	3H	singlet	-N=C-C<u>H</u>$_3$ (b)	-
3	2.37	3H	singlet	Ar-C<u>H</u>$_3$ (c)	-
4	2.44-2.55	1H	multiplet	-C<u>H</u>$_2$ (a)	-
5	2.77-2.96	2H	multiplet	-C<u>H</u>$_2$ (d)	-
6	4.72-4.76	1H	double doublet	-C<u>H</u> (e)	3.0 & 9.0
7	6.74-6.94	3H	multiplet	Ar-H (f,g,h)	-
8	7.17-7.20	2H	doublet	Ar-H (i)	8.1
9	7.72-7.75	2H	doublet	Ar-H (j)	8.4
10	9.40	1H	singlet	-CO-N<u>H</u> (k)	-

EXPANDED ^{1}H-NMR SPECTRUM

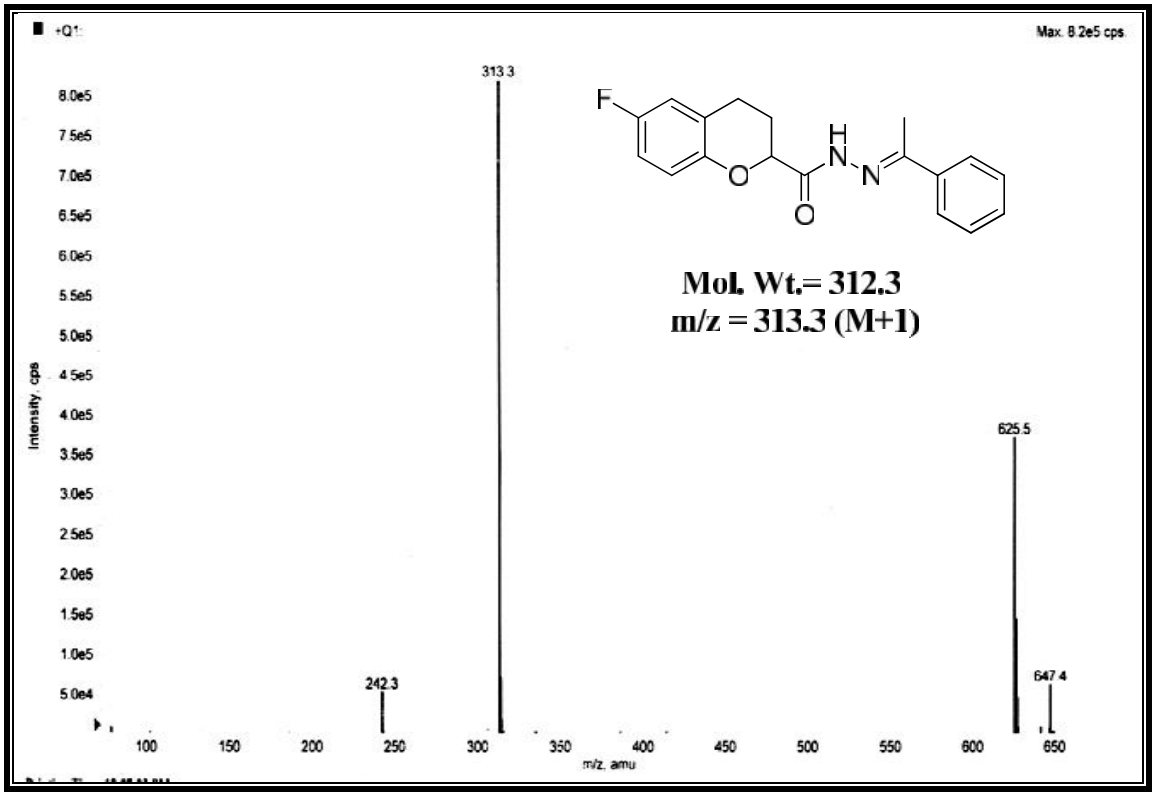




MASS SPECTRUM OF 6-FLUORO-*N*'-(1-(4-METHYLPHENYL)ETHYLIDENE)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



MASS SPECTRUM OF 6-FLUORO-*N*'-(1-PHENYLETHYLIDENE)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



EXPERIMENTAL

SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-(1-ARYLETHYLIDENE)-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRZIDE

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. 60 % Ethyl acetate in hexane was used as a mobile phase.

[A] SYNTHESIS OF 6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

See, Chapter-2, Part-I, Section-I, Experimental [B], Page no. 93.

[B] 6-FLUORO-*N*'-(1-(4-METHYLPHENYL)ETHYLIDENE)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

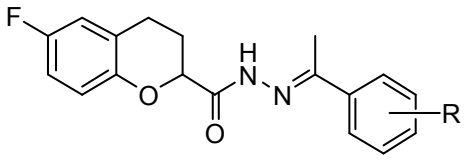
A mixture of 6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide (2.10 gm, 0.01 mol) and 4'-methyl acetophenone (1.34 gm, 0.01 mol) dissolved in ethanol (20 ml) in presence of catalytic amount of glacial acetic acid was refluxed for 9 hrs. The contents were cooled and poured in crused ice so solid precipitation was obtained, The product was filtered and dried. Isolated product was crystallized from ethanol. Yield: 81 %, M. P. 196-199 °C, ($C_{19}H_{19}FN_2O_2$; Required: C, 69.92; H, 5.87; N, 8.58; Found: C, 69.73; H, 5.69; N, 8.44 %).

Similarly, other *N*'-(1-arylethylidene)-6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrzide were **(6a-j)** prepared. The physical constants are recorded in **Table-6a**, Page no. 137.

[C] BIOLOGICAL SCREENING OF *N*'-(1-ARYLETHYLIDENE)-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRZIDE

Antimicrobial testing was carried out as described in Chapter-1**,** Section-I, Experimental Section-[C], Page no. 37. The results obtained from antimicrobial testing are recorded in **Table-6b,** Page no. 138.

TABLE6a: PHYSICAL CONSTANTS OF *N*'-ARYLMETHYLIDENE-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



Sr. No.	Substitution R	Molecular Formula/ Molecular Weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
6a	H	$C_{18}H_{17}FN_2O_2$ 312.34	181-182	85	69.22 69.03	5.49 5.36	8.97 8.81
6b	4-Me	$C_{19}H_{19}FN_2O_2$ 326.36	196-199	81	69.92 69.73	5.87 5.69	8.58 8.44
6c	4-F	$C_{18}H_{16}F_2N_2O_2$ 330.33	175-178	89	65.45 65.28	4.88 4.77	8.48 8.30
6d	4-Cl	$C_{18}H_{16}ClFN_2O_2$ 346.78	153-156	76	62.34 62.26	4.65 4.52	8.08 7.92
6e	3-Cl	$C_{18}H_{16}ClFN_2O_2$ 346.78	169-170	72	62.34 62.06	4.65 4.56	8.08 7.96
6f	2,4-$(Cl)_2$	$C_{18}H_{15}Cl_2FN_2O_2$ 381.23	188-191	66	56.71 56.43	3.97 3.84	7.35 7.22
6g	3-Br	$C_{18}H_{16}BrFN_2O_2$ 391.23	171-174	75	55.26 55.03	4.12 4.01	7.16 7.09
6h	4-OH	$C_{18}H_{17}FN_2O_3$ 328.34	192-195	83	65.84 65.51	5.22 5.13	8.53 8.39
6i	4-NH_2	$C_{18}H_{18}FN_3O_2$ 327.35	182-184	61	66.04 65.87	5.54 5.41	12.84 12.68
6j	2-NO_2	$C_{18}H_{16}FN_3O_4$ 357.34	204-206	88	60.50 60.36	4.51 4.43	11.76 11.69

TABLE 6b: BIOLOGICAL SCREENING OF *N*'-ARYLMETHYLIDENE-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	6a	250	500	250	500	250	500	500
2	6b	500	500	**62.5**	200	**100**	500	500
3	6c	250	**50**	250	250	**100**	250	250
4	6d	500	500	250	200	200	500	500
5	6e	**100**	500	500	500	500	>1000	>1000
6	6f	250	500	100	100	250	>1000	>1000
7	6g	250	250	250	200	200	1000	1000
8	6h	**100**	100	250	500	500	>1000	>1000
9	6i	200	500	250	250	500	1000	1000
10	6j	250	250	500	500	200	>1000	>1000

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted Schiff bases **6e** (R= 3-Cl) & **6h** (R= 4-OH) against *S.aureus,* **6c** (R= 4-F) against *S.pyogenus* and **6b** (R= 4-Me) against *E-coli* possess very good activity compared to ampicillin. While **6i** (R= 4-NH_2) against *S.aureus,* **6h** (R= 4-OH) against *S.pyogenus* and **6f** (R= 2,4-di Cl) against *E-coli* & *P.aeruginosa,* demonstrate moderate activity as compared to ampicillin. The remaining schiff bases exhibit moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted schiff bases **6b** (R= 4-Me) & **6c** (R= 4-F) display highly promissing activity against *C.albicans* as compared to greseofulvin. While **6d** (R= 4-Cl) & **6j** (R= 2-NO_2) against *C.albicans* and **6c** (R= 4-F) against *A.niger* & *A.clavatus,* possess moderate activity compare to standard drug. The remaining compounds show moderate to poor activity against all three bacterial species.

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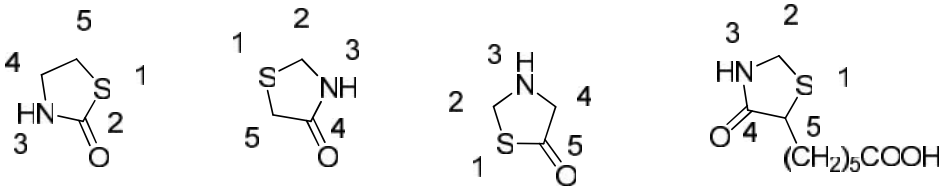
Chapter-4

STUDIES ON THIAZOLIDINONE DERIVATIVES

INTRODUCTION

Thiazolidinones, which belong to an important group of heterocyclic compounds, have been widely explored for their applications in the field of medicine. Thiazolidinones play a vital role due to their wide range of biological activities and industrial importance. Thiazolidinones, with a carbonyl group at position 2 in structure (1), position 4 in structure (2) and position 5 in structure (3) have been subjected to widespread study in the recent past.



1 2 3 4

4-Thiazolidinones are always being an attraction point for researchers because of its efficiency towards various pharmacological usages. Numerous gossips have appeared in the literatures which underscore their chemistry and use. The derivatives of 4-thiazolidinone nucleus have occupied a unique place in the field of medicinal chemistry due to wide range of biological activities like antibacterial, antitubercular, anticancer, anticonvulsant and antifungal.[1] They have interesting activity profiles mainly cox-1 inhibitors, inhibitors of bacterial enzyme, non nucleoside inhibitors of HIVRT and antihistaminic agents.[2] 4-thiazolidinones are derivatives of thiazolidinone with carbonyl group at the 4th position and formed by the attack of sulphur nucleophile on imine carbon followed by intramolecular cyclisation with elimination of water. Substituent in the 2, 3 and 5 positions may be varied, but the greatest different in structure and properties is exerted by the groups attached to carbon atom at the 2-position and to nitrogen atom at the 3-position. The cyclic structure was assigned after recognition of mercaptoacetic acid as a primary product of hydrolysis of 3-phenyl-2-phenylimino-4-thiazolidinones.[3]

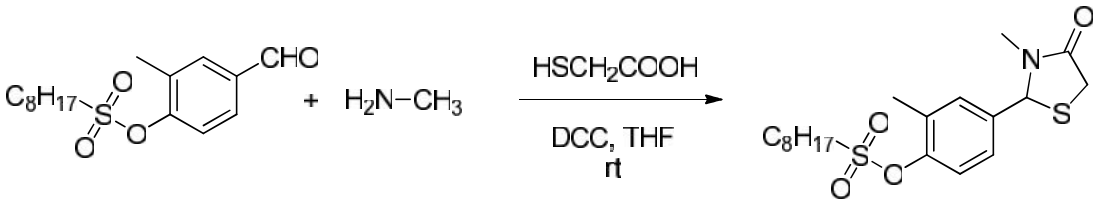
A well known antibiotic, actithiazic acid (4), isolated from a species of streptomyces shows specific in vitro activity against *M. tuberculosis*, but it is inactive in vivo probably due to antagonisation by biotin, bears the 4-thiazolidinone skeleton. A recent literature search revealed that 4–thiazolidinone derivatives may exhibit antibacterial[4,5], antituberculosis[6-8], antiviral[9-14] and anticancer[15-18] activities. According to Andres et al.[4], 4-thiazolidinones may be considered as phosphate bioisosteres and therefore inhibit the bacterial enzyme *Murb* which is involved in the biosynthesis of peptidoglycan layer of the cell wall[4]. In addition, some thiazolidinones were recently

reported as novel inhibitors of *mycobacterial rhamnose* synthetic enzymes[19]. This new approach is believed to be selective as rhamnose which is not found in humans, has been shown to be essential for mycobacterial cell wall synthesis[19].

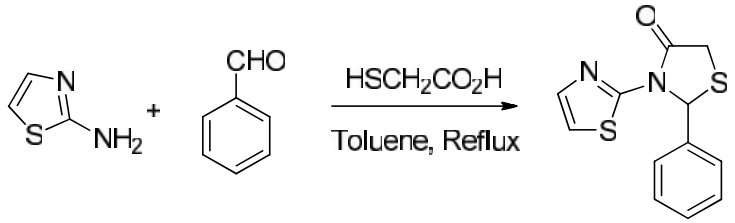
SYNTHETIC ASPECT

Synthesis of 4-thiazolidinones has been reported either by cyclisation of acyclic compounds or by interconversion among appropriately substituted thiazolidinone derivatives. Several methods for the preparation of 4-thiazolidinones are narrated in literature.[20-29]

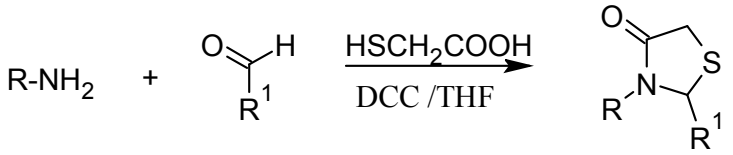
1. Hongyu Zhou et al.[30] have reported Microwave-Assisted Fluorous Synthesis of 4-thiazolidinone and generte 4-thiazolidinone libraries.



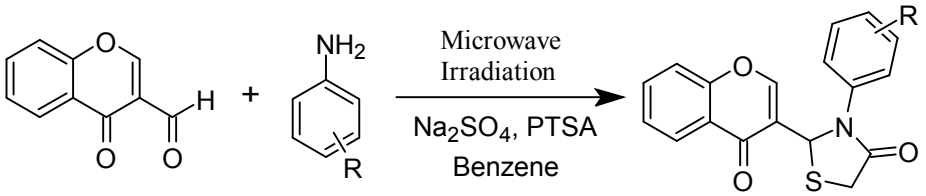
2. Ravindra K. Rawal et al.[31] designed and synthesized a series of 2-aryl-3-heteroaryl-1,3-thiazolidin-4-ones and evaluated for anti-HIV-1 RT activity.



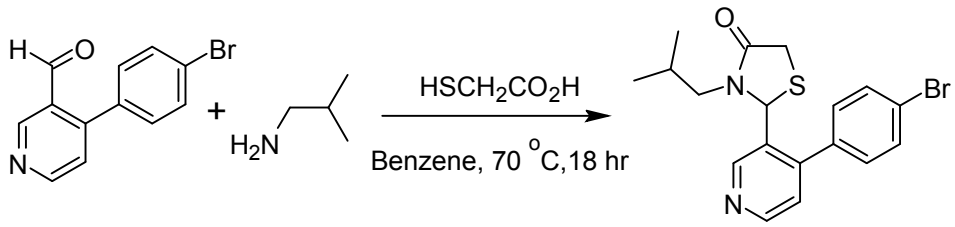
3. Tumul Srivastava et al.[32] have synthesized some new 4-thiazolidinones by DCC mediated three-component reaction of amine, aldehyde and mercaptoacetic acid.



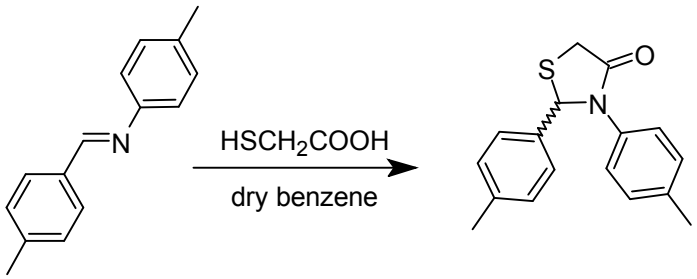
4. Zhong-Zheng Zhou et al.[33] have reported one-pot liquid-phase combinatorial synthesis of 2-(4-oxo-4H-1-benzopyran-3-yl)-4-thiazolidinones bearing diverse substituents at the 3-position under microwave irradiation.



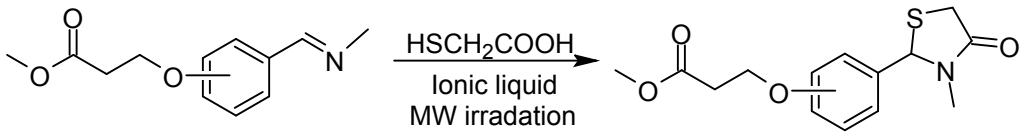
5. Christopher J. Hobbs et al.[34] have synthesized some new calcium channel blockers from the 'hit' structures 2-(3-bromo-4-fluorophenyl)-3-(2-pyridin-2-ylethyl)thiazolidin-4-one and its 2-[4-(4-bromophenyl)pyridin-3-yl]-3-isobutyl analogues.



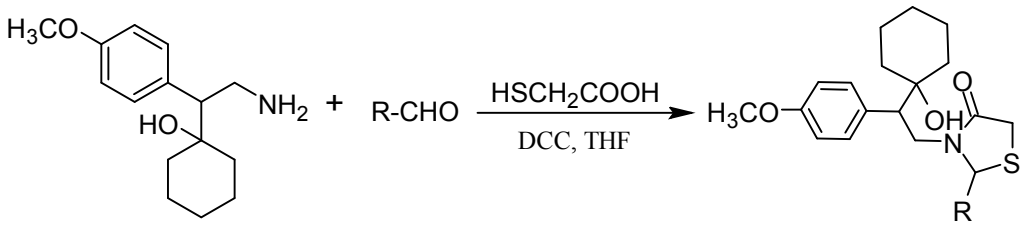
6. Adele Bolognese et al.[35] have been describe the thiazolidin-4-one formation. Mechanistic and synthetic aspects of the reaction between imines and mercaptoacetic acid under microwave and conventional heating.



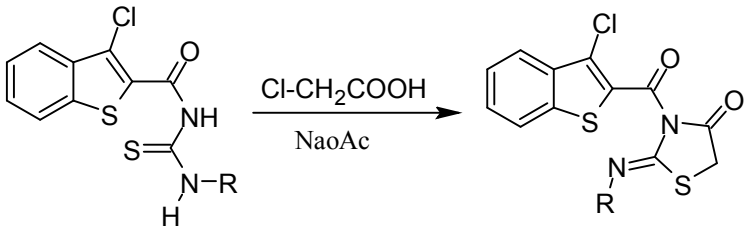
7. Joan Fraga-Dubreuil et al[36] have been explained efficient combination of task-specific ionic liquid and microwave dielectric heating applied to one-pot three component synthesis of a small library of 4-thiazolidinones.



8. Bioactive venlafaxine analogs such as 2,3-disubstituted-1,3-thiazolidinones have been synthesized and reported as antimicrobial agent by C. V. Kavitha and coworkers.[37]

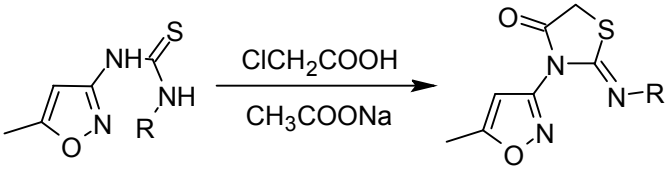


9. Denis R. St. Laurent et al.[38] have synthesized 4-thiazolidinone derivatives by the cyclization of unsymmetrical thiourea. H. S. Joshi and co-workers[39] have synthesized thiazolidinones bearing benzo[*b*]thiophenenucleus from *N*-rylaminothioxomethyl derivatives with chloroacetic acid in ethanol.



R= Aryl

10. M. Shrinivas et al.[40] have reported some new thiazolidinones from isoxazolyl thiourea on treatment with chloroacetic acid and fused anhydrous sodium acetate in ethanol.



R = Aryl

THERAPEUTIC IMPORTANCE

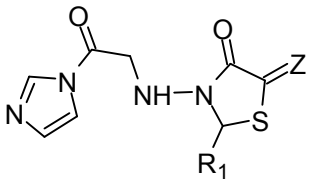
The thiazolidinone derivatives substituted at 2- and 3-position are associated with diverse biological activities which have been reported as under.

1. Analgesic[41]
2. Antibacterial[42,43]
3. Antidiabetic[44]
4. Antifungal[45-47]
5. Anti HIV and anti cancer[48]
6. Ant-microbial[49,50]
7. Antiulcer[51,52]
8. Anti-tumor[53]
9. Anti-tubuercular[54,55]
10. Anti-viral[56]
11. Anthelmintics[57,58]
12. Cardiovascular[59]
13. Herbicidal[60]
14. Hypnotic[61-63]
15. Insectidal[64]
17. Local anaesthetic[65]

K. Mogliaiah and Co-workers[66,67] isolated some 4-thiazolidinones derivativies and tested their antibacterial activity. H. Y. Hassan et. al.[68] has prepared

2-amino-4-thiazolidinones which have been found to possess antimicrobial activity. G. S. Gadaginamath et.al.[69] also prepared thiazolidinones derivatives, as antimicrobial agent.

R. S. Lodhi and co-workers[70] have been synthesized and studied antimicrobial, anti-inflammatory and analgesic activity of 4-thiazolidinone and arylidene derivatives (5).

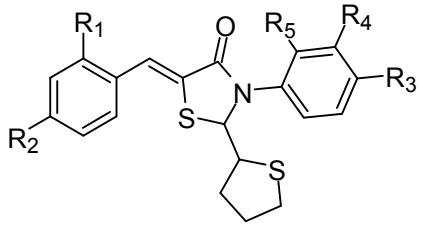


R = (Un) Substituted aryl
Z = H_2
Z = CH_2R_2
R_1 = Substituted aryl

(5)

G. Bhawana et. al.[71] have been synthesized thiazolidinone derivatives and compared their anti-inflammatory potency, ulcerogenic liability, cardiovascular and CNS effect. R. P. Pawar and co-workers[72] reported synthesis and in vitro antibacterial activity of some 4-thiazolidinone derivatives. In other study, some thiazolidinone derivatives have been found to be promising antibacterial agent.[73]

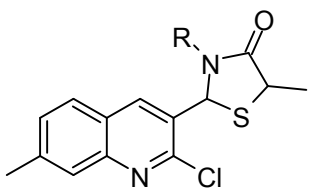
Morever, Albuquerque and co-workers[74] have prepared 4-thiazolidinones which show antidiabetic and antiinflammatory activity. Tagami and co-worker[75] have synthesized 4-thiazolidinone derivatives as allergy inhibitor. M. Siddique et. al.[76] have prepared substituted thiazolidinones (6) and reported their antibacterial, antifungal, antithyroid and amoebicidal properties.



R_1 = OH
R_2,R_4,R_5 = H
R_3 = Me

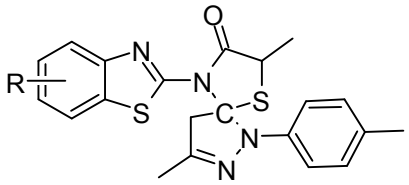
(6)

H. H. Parekh and et. al.[77] have been synthesized some new thiazolidinones (7) as antimicrobial agent.



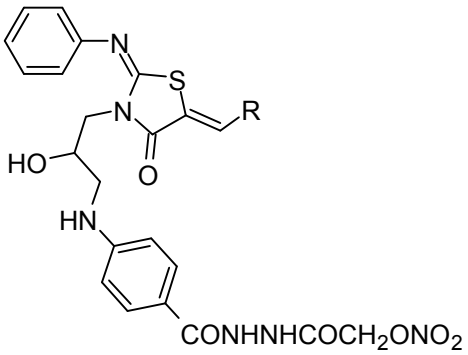
(7) R=Aryl

K. R. Desai et.al.[78] have been synthesized some new 4-thiazolidinones (8) as antimicrobial agent.



(8) R= Aryl

S. V. Bhandari et.al.[79] have been synthesized thiazolidinone derivatives (9) and tested for electrocardiographic, antiarrhythmic, vasorelaxing and antihypertensive activity as well as for in-vitro nitric oxide (NO) releasing ability.



(9) R= Substituted phenyl

In view of getting better therapeutic activities showed by 4-thiazolidinones, prompted us to synthesize 4-thiazolidinone derivatives, which have been described as under.

SECTION-I: SYNTHESIS AND BIOLOGICAL SCREENING OF *N*-(2-ARYL-4-OXOTHIAZOLIDIN-3-YL)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETAMIDE

SECTION-II: SYNTHESIS AND BIOLOGICAL SCREENING OF *N*-(2-ARYL-5-METHYL-4-OXOTHIAZOLIDIN-3-YL)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETAMIDE

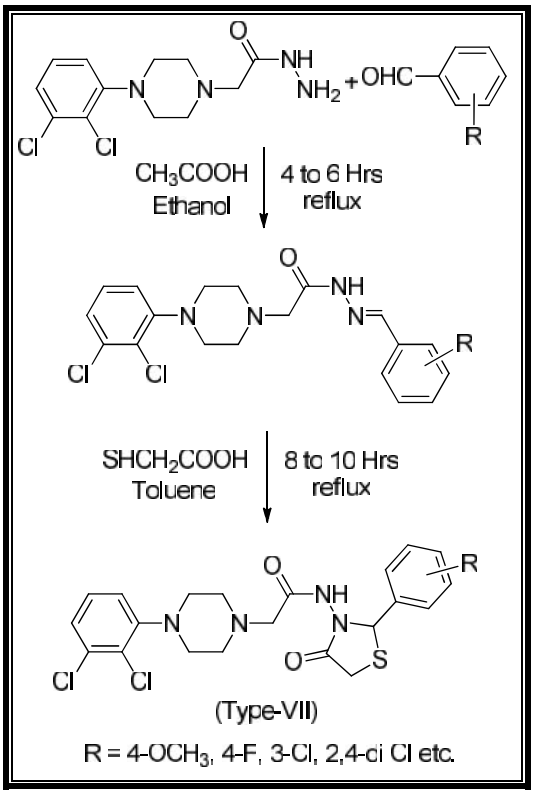
SECTION-I

SYNTHESIS AND BIOLOGICAL SCREENING OF *N*-(2-ARYL-4-OXOTHIAZOLIDIN-3-YL)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL) ACETAMIDE

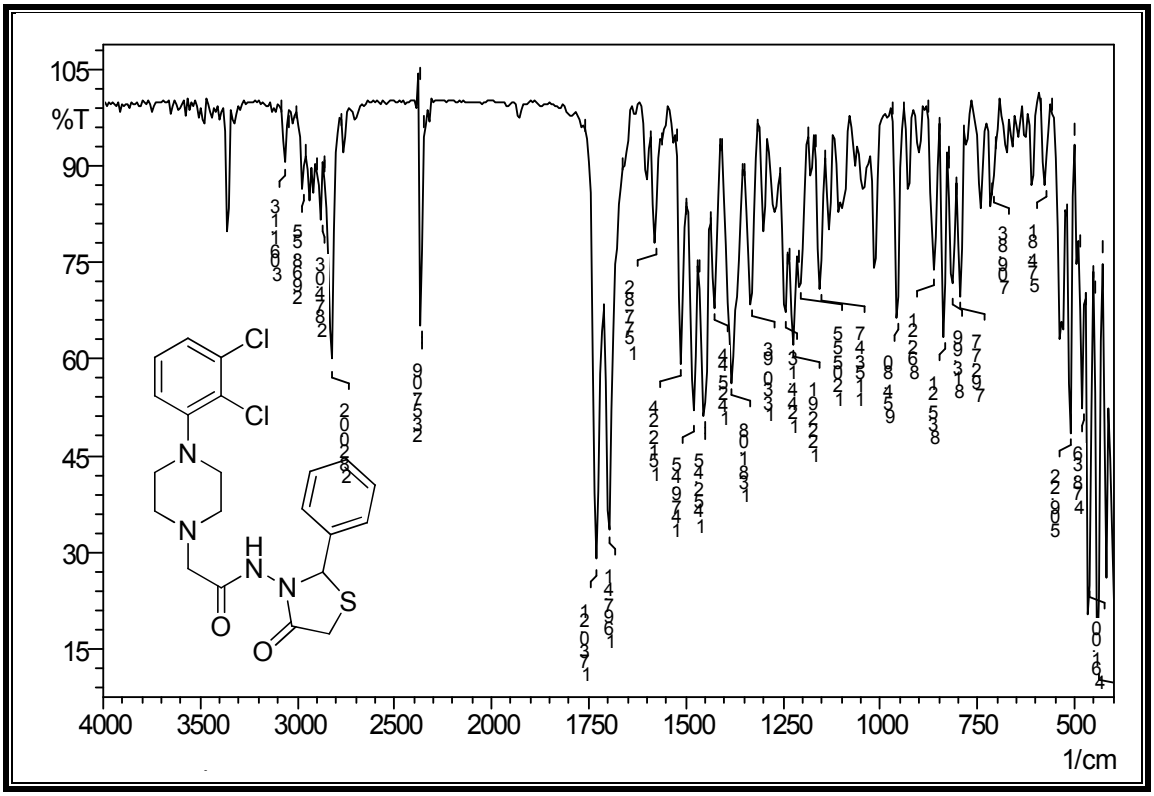
The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

REACTION SCHEME



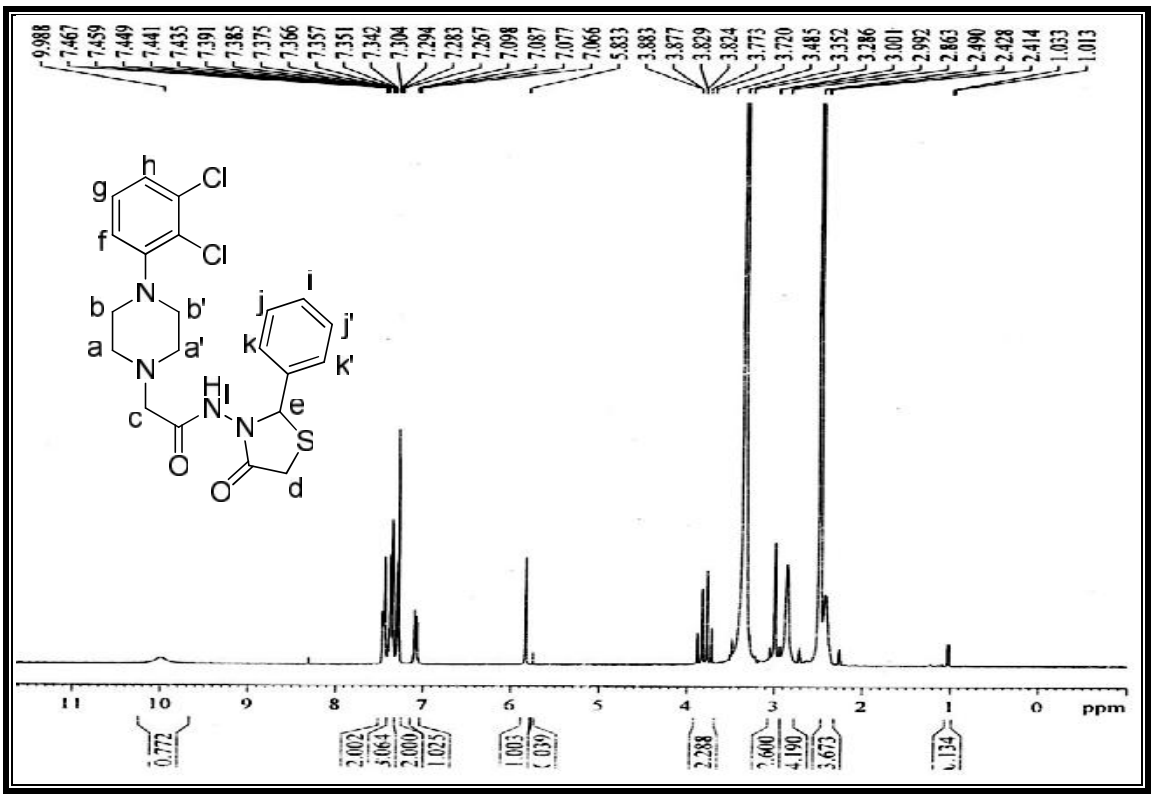
IR SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*-(4-OXO-2-PHENYLTHIAZOLIDIN-3-YL)ACETAMIDE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc).

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2968	2975-2920	80
	C-H str. (sym.)	2874	2880-2860	”
	C-H def. (asym.)	1452	1470-1435	”
	C-H def. (sym.)	1381	1395-1370	”
Aromatic	C-H str.	3061	3100-3000	”
	C=C str.	1512	1585-1480	”
	C-H i.p. def.	1153	1125-1090	”
	C-H o.o.p. def.	835	860-810	”
Thiazolidinone	C=O str.	1730	1760-1655	81
	C-N str.	1205	1220-1020	”
	C-S str.	709	750-600	”
Carbonyl Halide	C=O str.	1697	1700-1650	”
	C-Cl str.	792	850-650	”

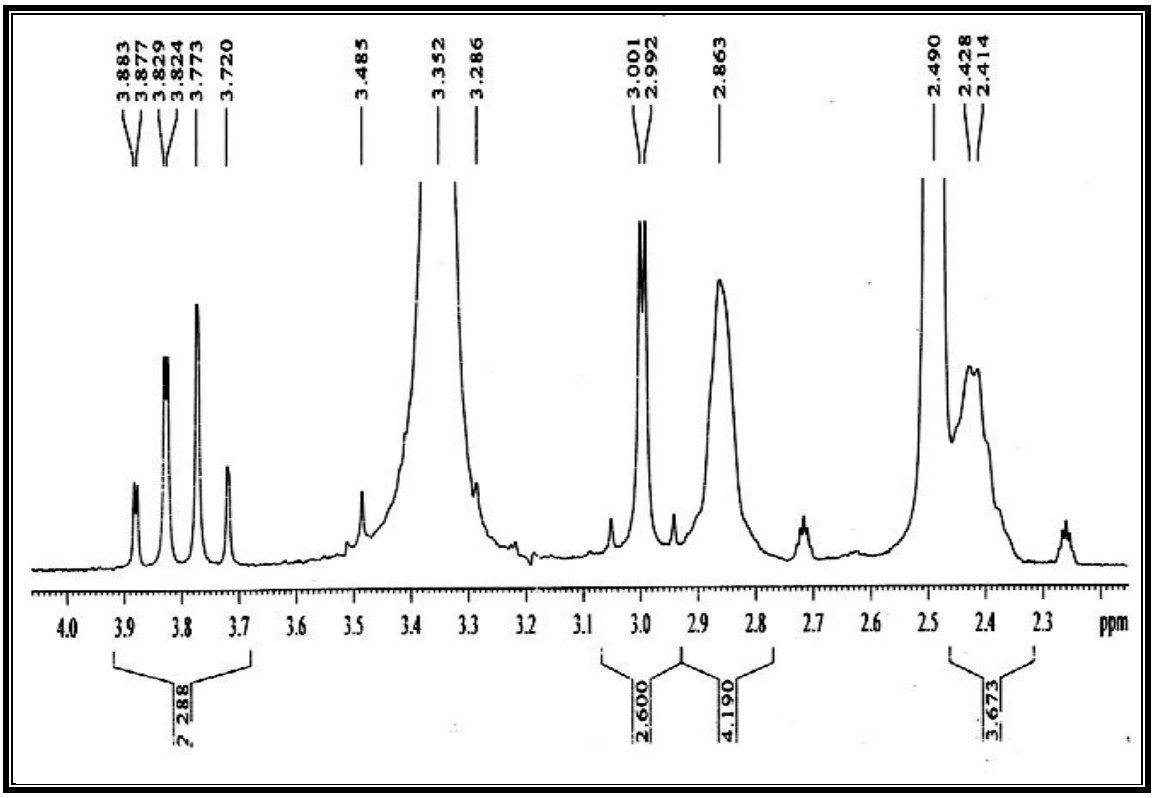
^{1}H-NMR SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*-(4-OXO-2-PHENYLTHIAZOLIDIN-3-YL)ACETAMIDE

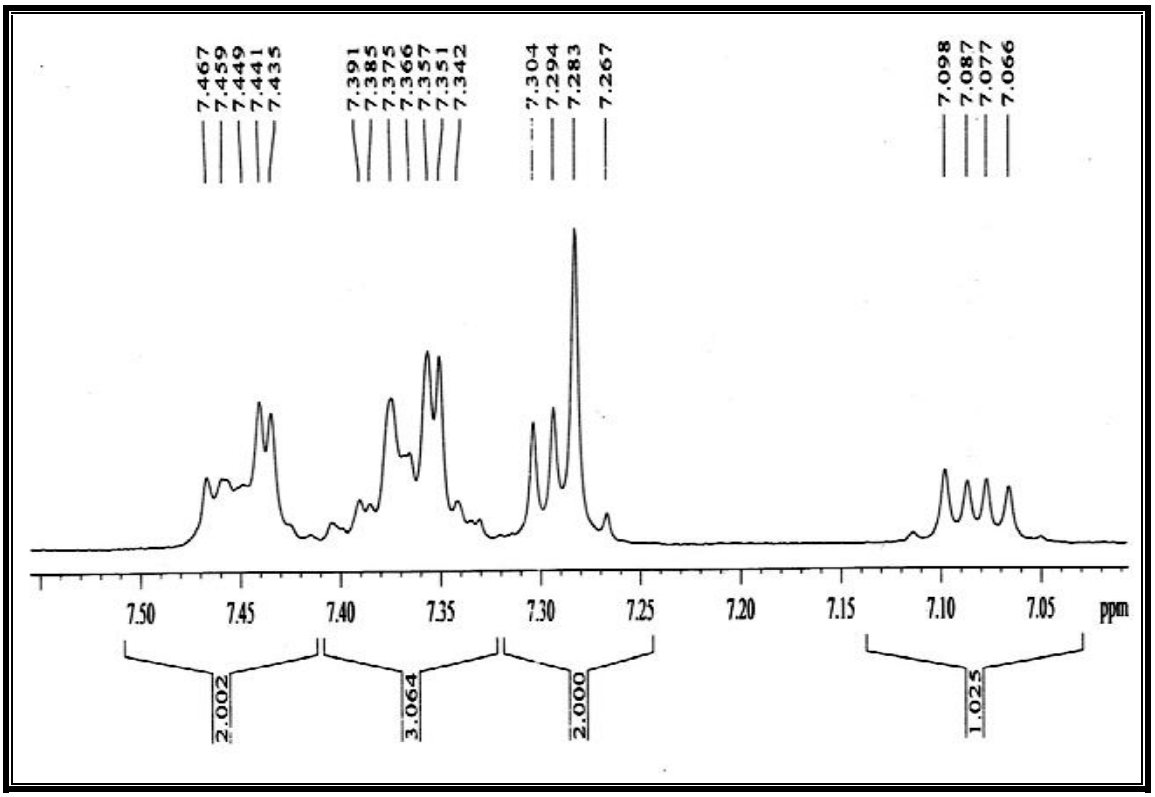


Internal Standard: TMS; Solvent: DMSO-d_6 Instrument: BRUKER Spectrometer (300MHz)

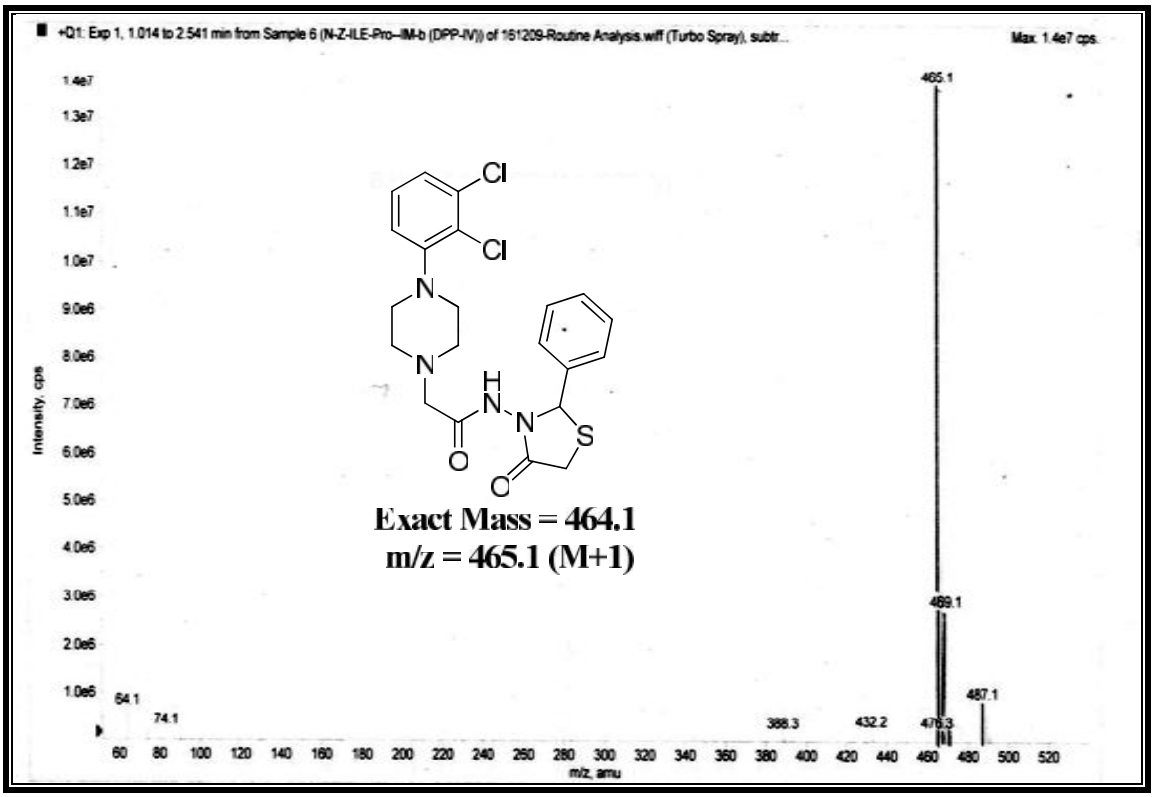
Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	2.41-2.2	4H	broad doublet	-C<u>H</u>$_2$ (a,a')	4.2
2	2.86	4H	broad singlet	-C<u>H</u>$_2$ (b,b')	-
3	3.72-3.88	2H	multiplet	-C<u>H</u>$_2$ (c)	-
4	2.99-3.00	2H	multiplet	-C<u>H</u>$_2$ (d)	-
5	5.83	1H	singlet	-C<u>H</u> (e)	-
6	7.06-7.09	1H	double doublet	Ar-H (f)	3.2 & 6.3
7	7.26-7.30	2H	multiplet	Ar-H (g,h)	-
8	7.34-7.39	3H	multiplet	Ar-H (i,j,j')	-
9	7.43-7.46	2H	multiplet	Ar-H (k,k')	-
10	9.98	1H	singlet	-CO-N<u>H</u> (l)	-

EXPANDED ^{1}H-NMR SPECTRUM

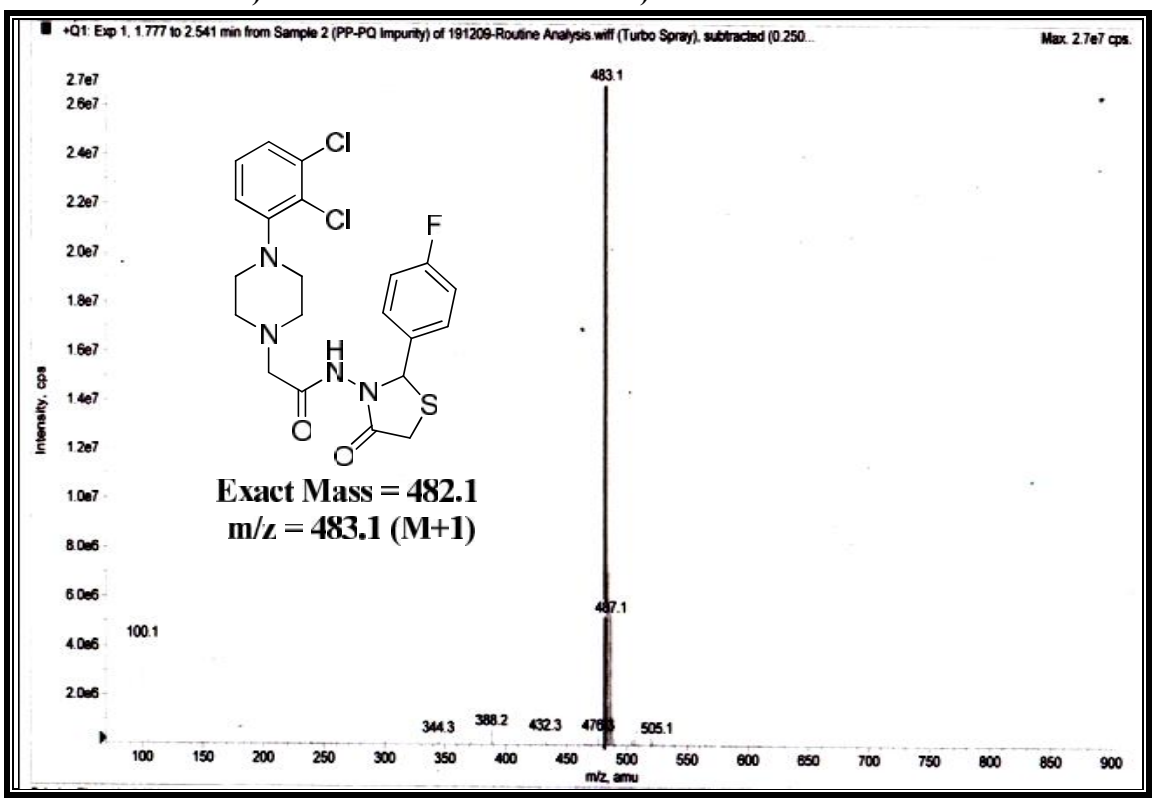




MASS SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*-(4-OXO-2-PHENYLTHIAZOLIDIN-3-YL)ACETAMIDE



MASS SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*-(2-(4-FLUOROPHENYL)-4-OXOTHIAZOLIDIN-3-YL)ACETAMIDE



EXPERIMENTAL

SYNTHESIS AND BIOLOGICAL SCREENING OF *N*-(2-ARYL-4-OXOTHIAZOLIDIN-3-YL)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL) ACETAMIDE

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. 5 % Methanol in chloroform was used as a mobile phase.

[A] SYNTHESIS OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*'-(PHENYLMETHYLIDENE)ACETOHYDRAZIDE

See, Chapter-3, Section-I, Experimental [B], Page no. 109.

[B] SYNTHESIS OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*-(4-OXO-2-PHENYLTHIAZOLIDIN-3-YL)ACETAMIDE

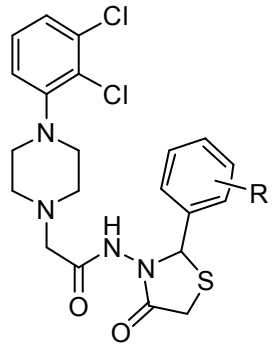
A mixture of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)-*N*'-(phenylmethylidene) acetohydrazide (3.91 gm, 0.01 mol) and thioglycolic acid (1.84 gm, 0.02 mol) in toluene (20 ml) was refluxed for 8 hrs. Seperated water from the reaction was continuesly removed by azeotropic distillation using a Dean-Stark separator. Progress of rhe reaction was monitered by TLC, after completion of reaction excess of toluene was distilled off and the resulting residue was partioned between ethyl acetate and saturated $NaHCO_3$ solution to remove unreacted thioglycolic acid, organic layer was washed with brine, dried over sodium sulphate, evaporated to give crude product. The crude product was recrystallized from mixture of isopropyl alcohol:hexane. Yield 65%, M. P. 135-138 ^{0}C. ($C_{21}H_{22}Cl_2N_4O_2S$; Required: C, 54.20; H, 4.76; N, 12.04 %; Found: C, 53.94; H, 4.73; N, 11.98 %).

Similarly, other *N*-(2-aryl-4-oxothiazolidin-3-yl)-2-(4-(2,3-dichlorophenyl) piperazin-1-yl)acetamide **(7a-j)** were prepared. The physical constants are recorded in **Table-7a**, Page no. 159.

[C] BIOLOGICAL SCREENING OF *N*-(2-ARYL-4-OXOTHIAZOLIDIN-3-YL)-2-(4-(2,3-DICHLOROPHENYL)PIPERA- ZIN-1-YL)ACETAMIDE

Antimicrobial testing was carried out as described in Chapter-1**,** Section-I, Experimental [C], Page no. 37. The results obtained from antimicrobial testing are recorded in **Table-7b**, Page no. 160

TABLE-7a: PHYSICAL CONSTANTS OF *N*-(2-ARYL-4-OXOTHIAZOLIDIN-3-YL)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETAMIDE



Sr. No.	Substitution R	Molecular Formula/ Molecular Weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
7a	H	$C_{21}H_{22}Cl_2N_4O_2S$ 465.40	135-138	65	54.20 53.94	4.76 4.73	12.04 11.98
7b	4-OMe	$C_{22}H_{24}Cl_2N_4O_3S$ 495.42	169-171	76	53.34 54.08	4.88 4081	11.31 11.22
7c	4-F	$C_{21}H_{21}Cl_2FN_4O_2S$ 483.39	144-146	71	52.18 51.96	4.38 4.29	11.59 11.45
7d	3-Cl	$C_{21}H_{21}Cl_3N_4O_2S$ 499.84	211-214	64	50.46 50.31	4.23 4.19	11.21 11.14
7e	2,4-$(Cl)_2$	$C_{21}H_{20}Cl_4N_4O_2S$ 534.29	188-190	54	47.21 47.03	3.77 3.71	10.49 10.36
7f	4-$N(Me)_2$	$C_{23}H_{27}Cl_2N_5O_2S$ 508.36	111-112	63	54.33 54.11	5.35 5.29	13.95 13.80
7g	2,5-$(OMe)_2$	$C_{23}H_{26}Cl_2N_4O_4S$ 525.45	158-161	58	52.57 52.41	4.99 4.92	10.66 10.57
7h	4-NO_2	$C_{21}H_{21}Cl_2N_5O_4S$ 510.39	148-151	73	49.42 49.24	4.15 4.11	13.72 13.64
7i	4-OH	$C_{21}H_{22}Cl_2N_4O_3S$ 481.40	170-171	62	52.39 52.21	4.61 4.56	11.64 11.61
7j	2-OH	$C_{21}H_{22}Cl_2N_4O_3S$ 481.40	182-185	55	52.39 52.24	4.61 4.51	11.64 11.55

TABLE-7b: BIOLOGICAL SCREENING OF *N*-(2-ARYL-4-OXOTHIAZOLIDIN-3-YL)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETAMIDE

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	7a	250	500	200	250	**250**	500	500
2	7b	500	500	250	100	500	250	250
3	7c	250	500	**62.5**	100	**200**	500	500
4	7d	**200**	250	250	200	500	1000	1000
5	7e	500	250	200	250	500	>1000	>1000
6	7f	500	250	500	250	**250**	1000	>1000
7	7g	500	500	250	200	500	500	500
8	7h	500	500	250	500	500	1000	1000
9	7i	250	100	100	250	500	500	1000
10	7j	500	250	250	200	500	1000	>1000

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted thiazolidinone **7d** (R= 3-Cl) against *S.aureus* and **7c** (R= 4-F) against *E-coli* possess excellent activity compared to ampicillin. While **7c** (R= 4-F) & **7i** (R= 4-OH) against *S.aureus,* **7i** (R= 4-OH) against *S.pyogenus* & *E-coli* and **7b** (R= 4-OMe) & **7c** (R= 4-F) against *P.aeruginos,* exhibit moderate activity as compared to ampicillin. The remaining compounds demonstrate moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted thiazolidinones **7a** (R= -H), **7c** (R= 4-F) & **7f** (R= 4-$N(Me)_2$) show highly promissing activity against *C.albicans* while **7b** (R= 4-OMe) display moderate activity against *A.niger* & *A.clavatus* as compare to greseofulvin. The remaining compounds show moderate to poor activity against all three bacterial species.

SECTION-II

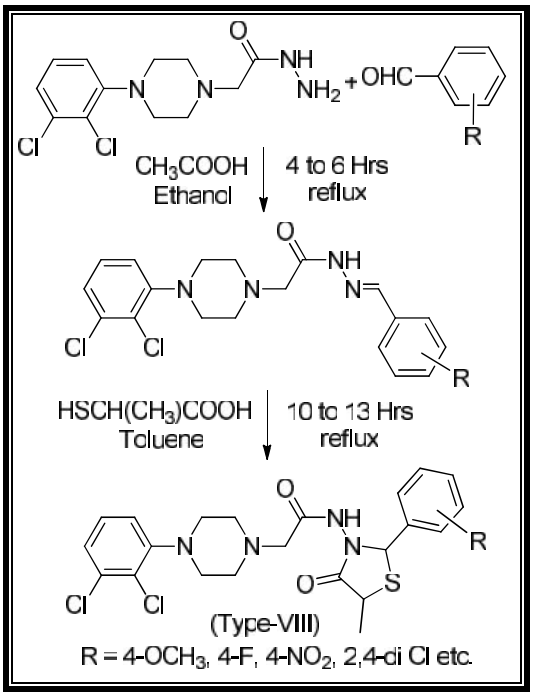
SYNTHESIS AND BIOLOGICAL SCREENING OF *N*-(2-ARYL-5-METHYL-4-OXOTHIAZOLIDIN-3-YL)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL) ACETAMIDE

4-Thiazolidinone derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activities, such as significant *in vitro* activity against DNA and RNA viruses including polio viruses, hypnotic, sedative, analgesic, diuretic, antitubercular, anticonvulsant, antifungal, antibacterial activity etc. These valid observations led us to synthesize some new 4-thiazolidinone derivatives. 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)-*N*-(5-methyl-4-oxo-2-arylthiazolidin-3-yl)acetamide of type (VIII) have been synthesized by the cyclization of *N*'-phenylmethylidene-2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide of Type (III) with thiolactic acid.

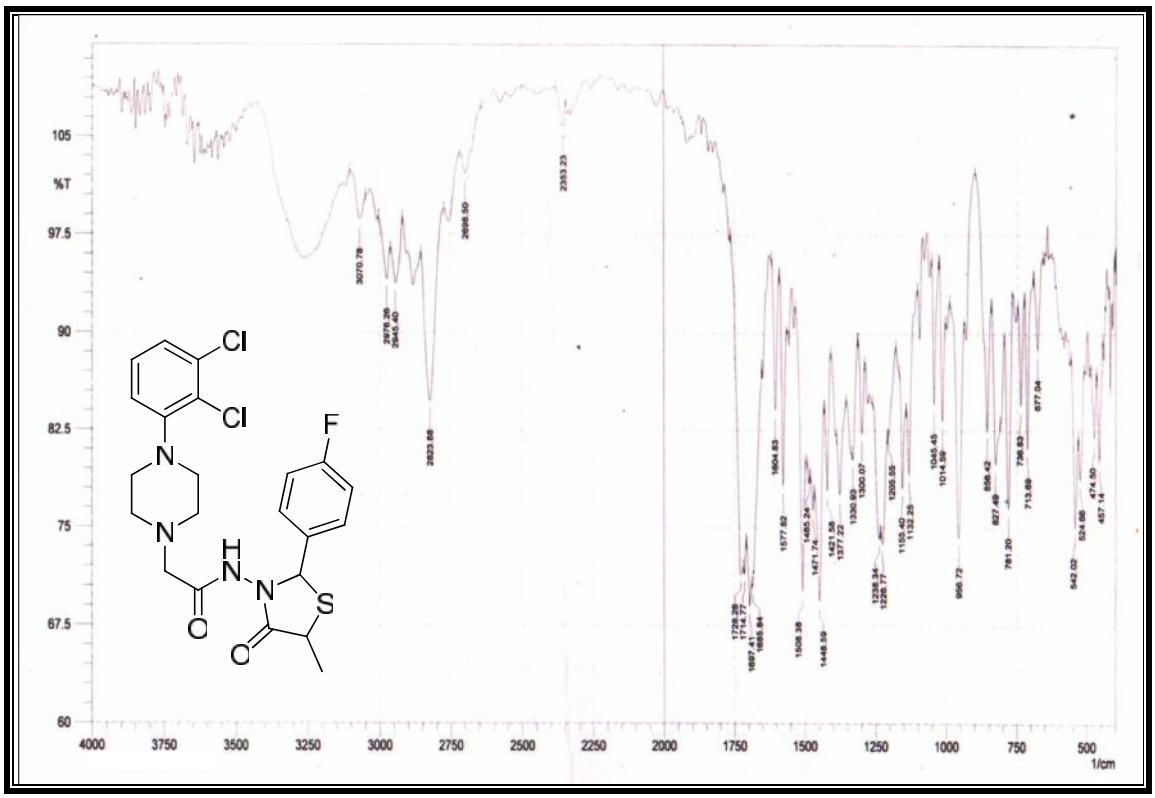
The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

REACTION SCHEME



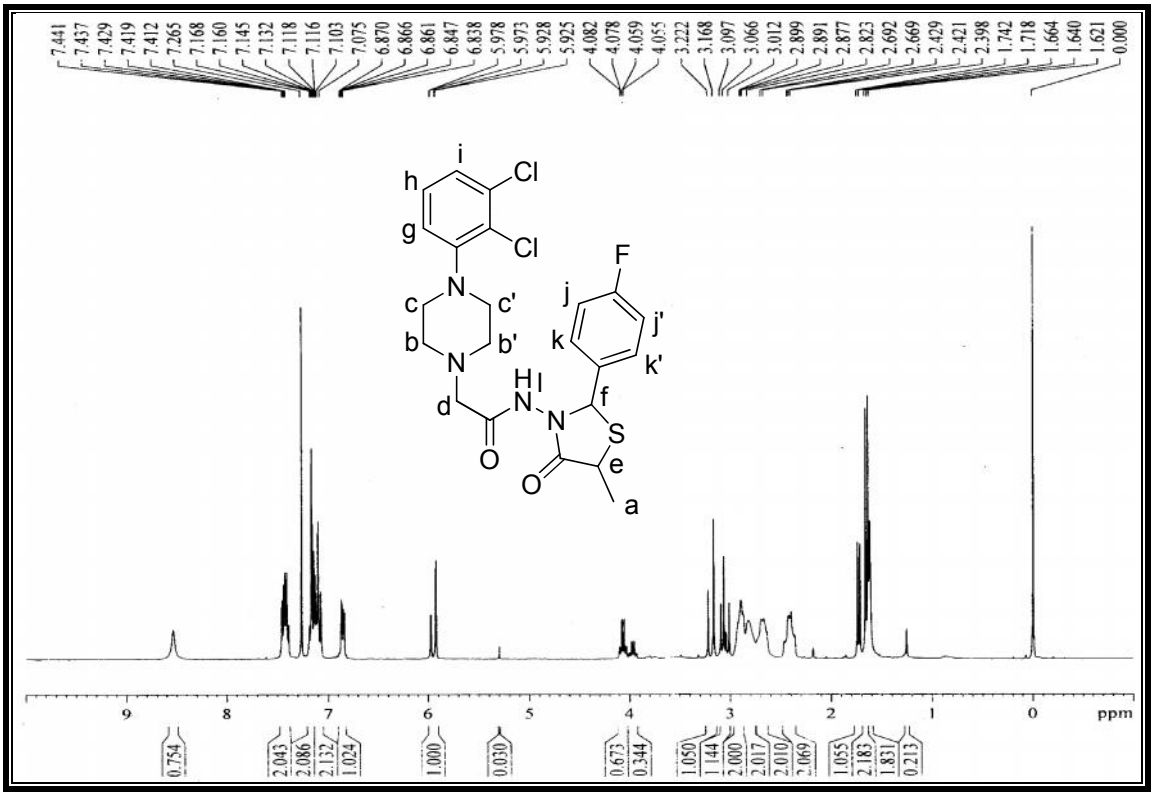
IR SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*-(2-(4-FLUOROPHENYL)-5-METHYL-4-OXOTHIAZOLIDIN-3-YL)ACETAMIDE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2945	2975-2920	80
	C-H str. (sym.)	2823	2880-2860	”
	C-H def. (asym.)	1448	1470-1435	”
	C-H def. (sym.)	1377	1395-1370	”
Aromatic	C-H str.	3070	3100-3000	”
	C=C str.	1508	1585-1480	”
	C-H i.p. def.	1132	1125-1090	”
	C-H o.o.p. def.	856	860-810	”
Thiazolidinone	C=O str.	1728	1760-1655	81
	C-N str.	1155	1220-1020	”
	C-S str.	713	750-600	”
Carbonyl Halide	C=O str.	1697	1700-1650	”
	C-Cl str.	781	850-650	”

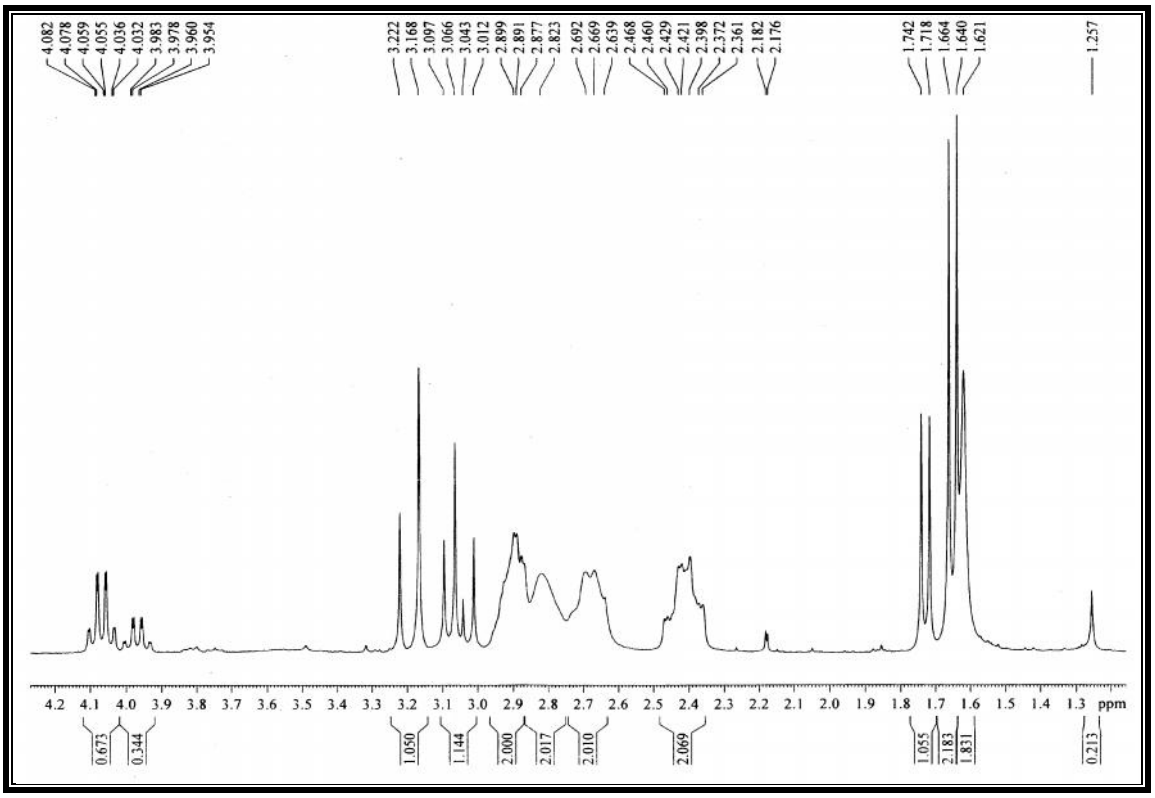
^{1}H-NMR SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-N-(2-(4-FLUOROPHENYL)-5-METHYL-4-OXOTHIAZOLIDIN-3-YL)ACETAMIDE

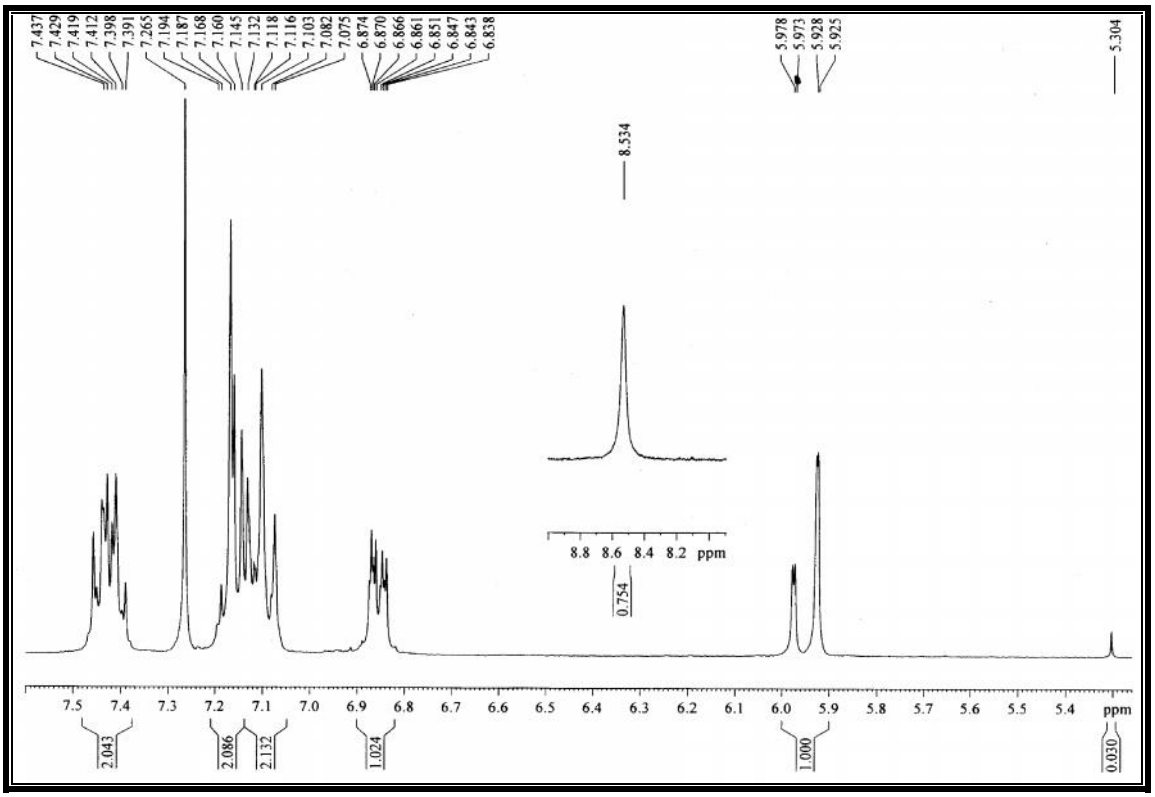


Internal Standard: TMS; Solvent: DMSO-d_6 Instrument: BRUKER Spectrometer (300MHz)

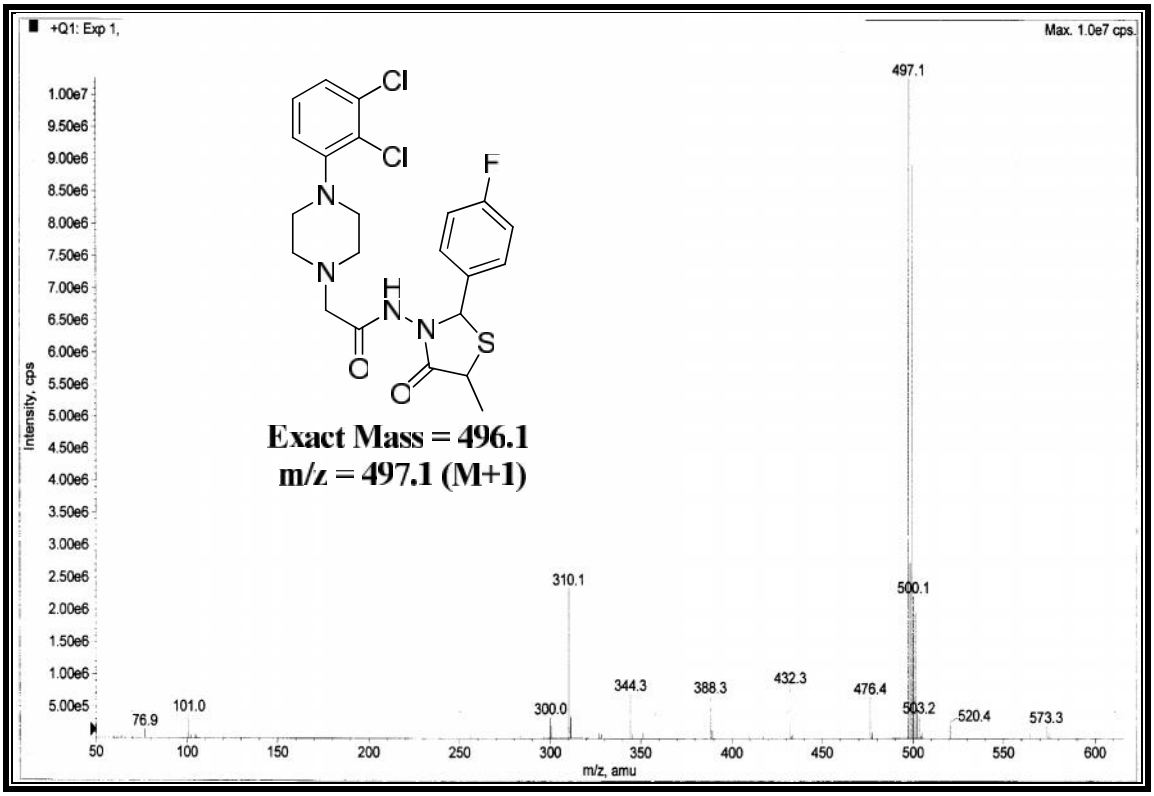
Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	1.64-1.66	3H	doublet	-C<u>H</u>$_3$ (a)	7.2
2	2.36-2.69	4H	multiplet	-C<u>H</u>$_2$ (b,b')	-
3	2.82-2.89	4H	multiplet	-C<u>H</u>$_2$ (c,c')	-
4	3.01-3.22	2H	multiplet	-C<u>H</u>$_2$ (d)	-
5	4.03-4.08	1H	quartet	-C<u>H</u> (e)	-
6	5.92	1H	doublet	-C<u>H</u> (f)	0.9
7	6.83-6.87	1H	multiplet	Ar-H (g)	-
8	7.07-7.19	4H	multiplet	Ar-H (h,i,j,j')	-
9	7.39-7.43	2H	multiplet	Ar-H (k,k')	-
10	8.53	2H	singlet	-CO-N<u>H</u> (l)	-

EXPANDED ^{1}H-NMR SPECTRUM

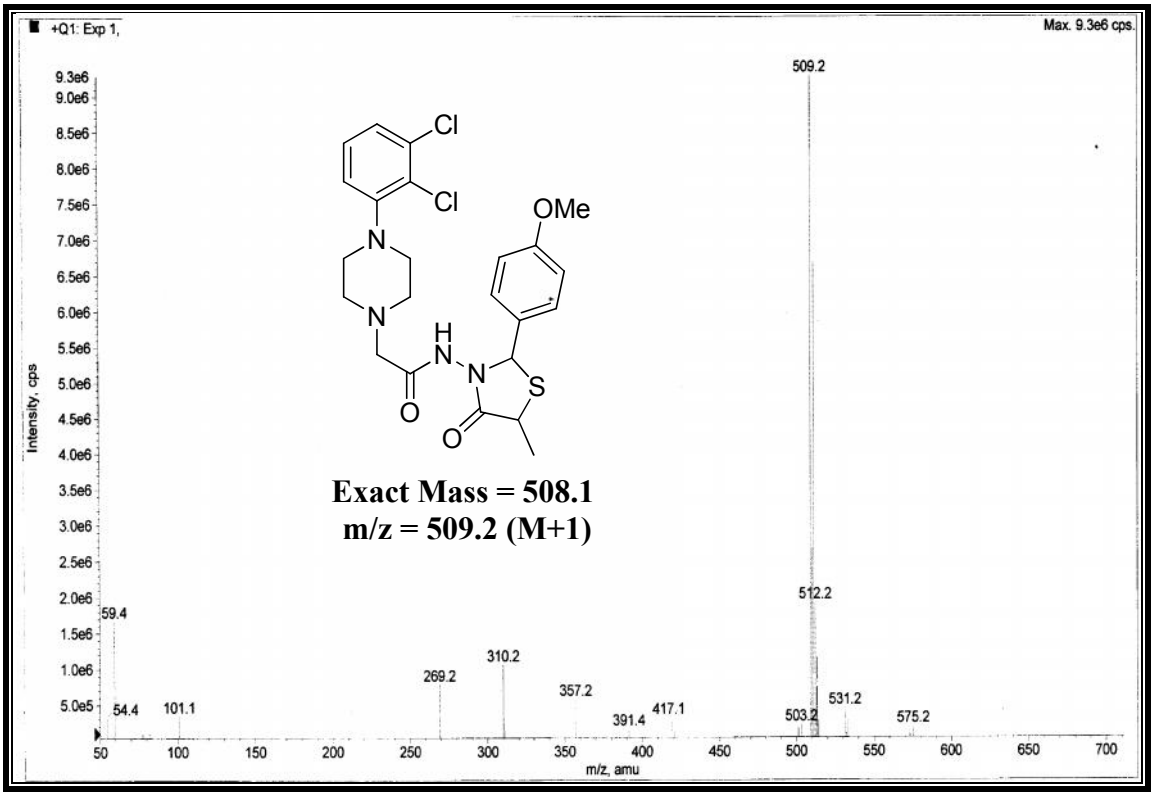




MASS SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-N-(2-(4-FLUOROPHENYL)-5-METHYL-4-OXOTHIAZOLIDIN-3-YL)ACETAMIDE



MASS SPECTRUM OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-N-(2-(4-METHOXYPHENYL)-4-OXOTHIAZOLIDIN-3-YL)ACETAMIDE



EXPERIMENTAL

SYNTHESIS AND BIOLOGICAL SCREENING OF *N*-(2-ARYL-5-METHYL-4-OXOTHIAZOLIDIN-3-YL)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL) ACETAMIDE

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. 5 % Methanol in chloroform was used as a mobile phase.

[A] SYNTHESIS OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*'-((4-FLUOROPHENYL)METHYLIDENE)ACETOHYDRAZIDE

See, Chapter-3, Section-I, Experimental [B], Page no. 109.

[B] SYNTHESIS OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-N-(2-(4-FLUOROPHENYL)-5-METHYL-4-OXOTHIAZOLIDIN-3-YL)ACETA-MIDE

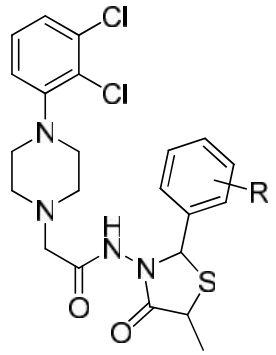
A mixture of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)-*N*'-((4-fluorophenyl) methylidene)acetohydrazide (4.09 gm, 0.01 mol) and thiolactic acid (2.12 gm, 0.02 mol) in toluene (20 ml) was refluxed for 12 hrs. Seperated water from the reaction was continuesly removed by azeotropic distillation using a Dean-Stark separator. Progress of rhe reaction was monitered by TLC, after completion of reaction excess of toluene was distilled off and the resulting residue was partioned between ethyl acetate and saturated $NaHCO_3$ solution to remove unreacted thioglycolic acid, organic layer was washed with brine, dried over sodium sulphate, evaporated to give crude product. The crude product was recrystallized from mixture of isopropyl alcohol and hexane. Yield 68%, M. P. 132^0C. ($C_{22}H_{23}Cl_2FN_4O_2S$; Required: C, 53.12; H, 4.66; N, 11.26; Found: C, 52.89; H, 4.52; N, 11.20 %).

Similarly, other 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)-*N*-(2-aryl-5-methyl-4-oxothiazolidin-3-yl)acetamide **(8a-j)** were prepared. The physical constants are recorded in **Table-8a**, Page no. 169

[C] BIOLOGICAL SCREENING OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)-*N*-(2-ARYL-5-METHYL-4-OXOTHIAZOLIDIN-3-YL) ACETAMIDE

Antimicrobial testing was carried out as described in Chapter-1**,** Section-I, Experimental Section-[C], Page no. 37. The results obtained from antimicrobial testinng are recorded in **Table-8b,** Page no. 170.

TABLE-8a: PHYSICAL CONSTANTS OF *N*-(2-ARYL-5-METHYL-4-OXOTHIAZOLIDIN-3-YL)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETAMIDE



Sr. No.	Substitution R	Molecular Formula/ Molecular Weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
8a	H	$C_{22}H_{24}Cl_2N_4O_2S$ 479.42	143-145	68	55.12 51.91	5.05 5.00	11.69 11.61
8b	4-OMe	$C_{23}H_{26}Cl_2N_4O_3S$ 509.45	152-155	62	54.22 54.03	5.14 5.07	11.00 10.92
8c	4-F	$C_{22}H_{23}Cl_2FN_4O_2S$ 497.41	129-131	58	53.12 52.89	4.66 4.52	11.26 11.20
8d	3-Cl	$C_{22}H_{23}Cl_3N_4O_2S$ 513.87	180-182	66	51.42 51.24	4.51 4.42	10.90 10.82
8e	2,4-$(Cl)_2$	$C_{22}H_{22}Cl_4N_4O_2S$ 548.31	164-167	51	48.49 48.32	4.04 3.99	10.22 10.13
8f	4-$N(Me)_2$	$C_{24}H_{29}Cl_2N_5O_2S$ 522.49	78-81	57	55.17 51.91	5.59 5.52	13.40 13.31
8g	2,5-$(OMe)_2$	$C_{24}H_{28}Cl_2N_4O_4S$ 539.47	171-173	49	53.43 53.27	5.23 5.14	10.39 10.25
8h	4-NO_2	$C_{22}H_{23}Cl_2N_5O_4S$ 524.42	135-137	63	50.39 50.16	4.42 4.33	13.35 13.23
8i	4-OH	$C_{22}H_{24}Cl_2N_4O_3S$ 495.42	154-156	52	53.34 53.15	4.88 4.85	11.31 11.24
8j	2-OH	$C_{22}H_{24}Cl_2N_4O_3S$ 495.42	166-168	45	53.34 53.21	4.88 4.79	11.31 11.21

TABLE-8b: BIOLOGICAL SCREENING OF *N*-(2-ARYL-5-METHYL-4-OXOTHIAZOLIDIN-3-YL)-2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL) ACETAMIDE

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	8a	500	500	250	250	**250**	500	1000
2	8b	500	500	250	500	500	1000	1000
3	8c	**100**	500	**62.5**	100	**250**	500	>1000
4	8d	250	100	200	250	500	250	200
5	8e	500	250	500	500	500	500	>1000
6	8f	500	250	500	250	500	>1000	1000
7	8g	250	500	200	500	500	1000	500
8	8h	500	250	500	250	500	1000	500
9	8i	500	**50**	200	100	500	250	200
10	8j	500	200	100	200	1000	500	500

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted thiazolidinone **8c** (R= 4-F) against *S.aureus*, **8i** (R= 4-OH) against *S.pyogenus* and **8c** (R= 4-F) against *E-coli* possess very good activity compared to ampicillin. While **8d** (R= 3-Cl) against *S.pyogenus,* **8j** (R= 2-OH) against *E-coli* and **8c** (R= 4-F) & **8i** (R= 4-OH) against *P.aeruginos,* exhibit moderate activity as compared to ampicillin. The remaining compounds show moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted thiazolidinones **8a** (R= -H) & **7c** (R= 4-F) display excellent activity against *C.albicans* while **8d** (R= 3-Cl) & **8i** (R= 4-OH) possess moderate activity against *A.niger* & *A.clavatus* as compare to greseofulvin. The remaining compounds demonstrate moderate to poor activity against all three bacterial species.

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Chapter-5

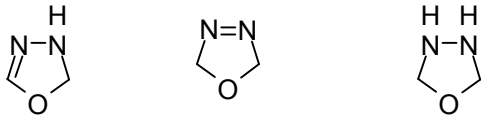
STUDIES ON OXADIAZOLE DERIVATIVES

INTRODUCTION

Oxadiazoles are five-membered aromatic heterocycles (having one oxygen and two nitrogen atom in five member ring system) with great utility in synthetic chemistry, medicinal chemistry and materials science. In the field of medicinal chemistry, oxadiazole derivatives are utilized as ester or amide surrogates (bioisosteres) [1] and they also having good fluorescence properties.[2] It is well documented that oxadiazoles are of four types which are numbered by designating the hetero atoms at particular position.

(1) (2) (3) (4)

1,3,4-Oxadiazole is a heterocyclic molecule with oxygen atom at 1 and two nitrogen atoms at 3 and 4 position. 1,3,4-Oxadiazoles belong to an important group of heterocyclic compounds having –N=C-O- linkage. 1,3,4-oxadiazole (4) is a thermally stable aromatic heterocycle and exist in partially reduced forms; 2,3-dihydro-1,3,4-oxadiazole(1,3,4-oxadiazoline) (5) and 2,5-dihydro-1,3,4-oxadiazole (1,3,4-oxadiazoline) (6) depending on the position of the double bond. The completely reduced form of the 1,3,4-oxadiazole is known as 2,3,4,5-tetrahydro-1,3,4-oxadiazole (1,3,4-oxadiazolidine) (7).



(5) (6) (7)

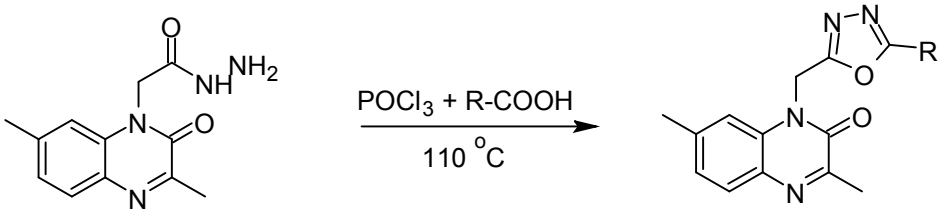
They have been known for about 80 years, it is only in the last decade that investigations in this field have been intensified. This is because of large number of applications of 1,3,4-oxadiazoles in the most diverse areas viz. drug synthesis, dye stuff industry, heat resistant materials, heat resistant polymers and scintillators. Reviews of the relevant literature prior to 1965 are available.[3]

SYNTHETIC ASPECT

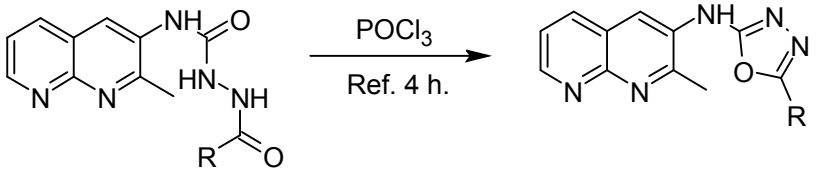
To the best of our knowledge, limited methodology exists for the synthesis of 1,3,4-oxadiazoles. Typically, a two step protocol is utilized where acylhydrazide and carboxylic acid derivatives are coupled under amide bond forming conditions and the resulting diacylhydrazide intermediate isolated and cycliszed using standard reagents such as $Ph_3P/CCl_4/Et_3N$,[4] Burgess reagent,[5] H_2SO_4,[6] $SOCl_2$,[7] P_2O_5,[8] $POCl_3$[9] and triflic

anhydride.[10] A few one pot methods have been reported in the literature. Different methods for the synthesis have been cited in literature.[11-16]

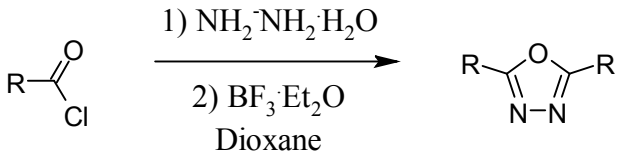
1. S. Wagle et al.[17] have synthesized oxadiazoles by the reaction of hydrazide with aromatic acid in presence of $POCl_3$.



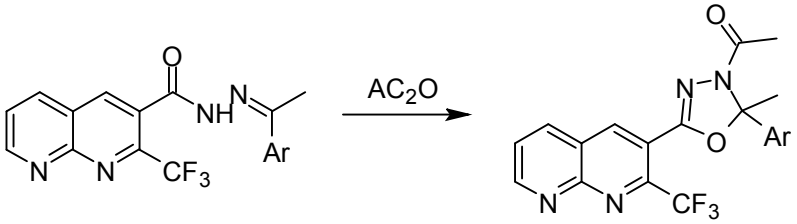
2. D. Ramesh and B. Sreenivasan[18] have synthesized 1,3,4-oxadiazoles from semicarbazide derivative in presense of $POCl_3$.



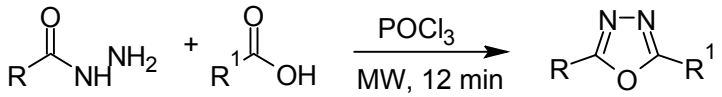
3. V. K. Tandon and R. B. Choor[19] described a simple, efficient and convenient synthesis of 1,3,4-oxadiazoles using $BF_3{\cdot}Et_2O$ as a cyclodehydrating agent.



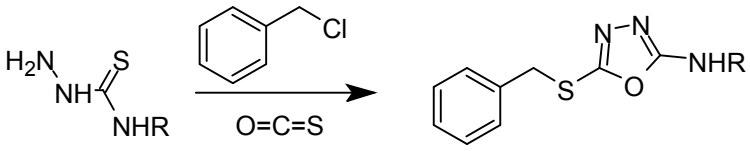
4. K. Mogilaiah & B. Sakram[20] have prepared 1,3,4-oxadiazole from Acetophenone-2-trifluoromethyl-1,8-naphthyridine-3-carbonyl hydrazone in presence of acetic anhydride.



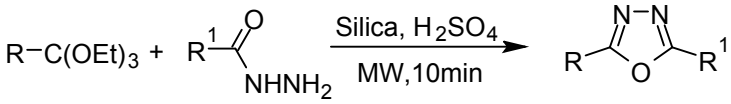
5. Yu Yuve[21] have reported microwave-assisted synthesis protocol with 91 % yield.



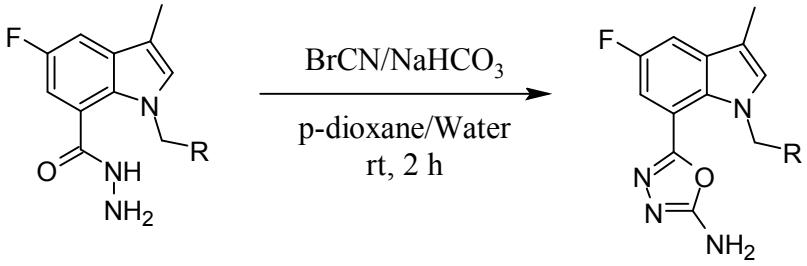
6. M. Chande et al.[22] reported the reaction of thiosemicarbazide ($RNHCSNHNH_2$) with carbon oxysulfide and benzyl chloride, which yields amino-oxadiazolyl thioethers.



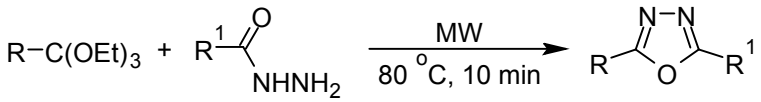
7. Silica sulfuric acid catalyst used for the rapid and ecofriendly synthesis of 1,3,4-oxadiazoles at ambient temperature by M. Dabiri et al.[23]



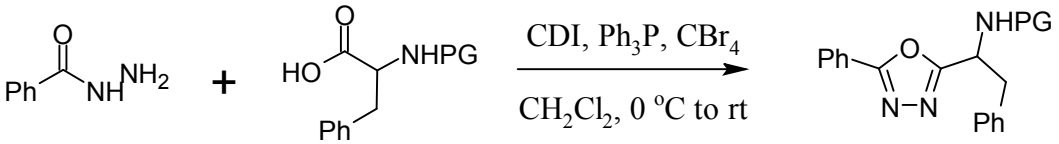
8. A. L. Polozov et al.[24] prepared 2-amino-1,3,4-oxadiazoles by reacting hydrazide derivative with cyanogen bromide in aqueous p-dioxane in the presence of sodium carbonate.



9. Solvent free microwave mediated one pot synthesis of 1,3,4-oxadiazoles were reported by V. Polshettiwar.[25]



10. H. A. Rajakapse et al.[26] reported a mild and efficient one pot synthesis of 1,3,4-oxadiazoles from acyl hydrazides and carboxylic acids by using CDI/Ph_3P/CBr_4.



THERAPEUTIC IMPORTANCE

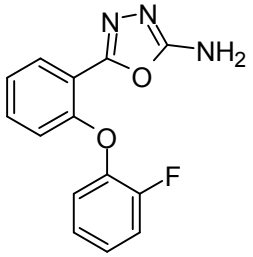
1,3,4-Oxadiazoles constitute one of the most active class of compounds possessing diverse pharmacological and microbiological activity. 1,3,4-Oxadiazole derivatives have been examined for various pharmacological activities, which have been summarized as under.

1. Antibacterial[27]
2. Antifungal[28]
3. Antiinflammatory[29]

4. Analgesic[30]
5. Antiviral and anticancer[31]
6. Antihypertensive[32]
7. Anticonvulsant[33]
8. Antiproliferative[34]
9. Cardiovascular[35]
10. Hypoglycemic[36]
11. Hypnotic and Sedative[37]
12. MAO inhibitor[38]
13. Insecticidal[39]

K. Mogilaiah and K. Vidya synthesized some 1,3,4-oxadiazole derivatives and screened for antibacterial activity.[40] S. R. Bishnoi et al.[41] have screened oxadiazoles for their antimicrobial activity. A. El-Azzouny et al.[42] have synthesized 1,3,4-oxadiazole derivatives and evaluated for their analgesic, anti-inflammatory, ulcerogenic effects and inhibitory activity on plasma prostaglandin E_2 (PGE_2) Level. S. V. Bhandari et al.[43] have reported 1,3,4-oxadiazoles for their anti-inflammatory activity.

Song Cao et al.[44] have investigated some oxadiazoles possessing insecticidal activity. G. V. Suresh Kumar et al.[45] have discovered oxadiazole derivatives and reported their antimycobacterial activity. Ali Almasired et al.[46] have prepared 1,3,4-oxadiazoles of type (8) as anticonvulsant agent. Meria Grazia Mamolo et al.[47] have synthesized 3-substituted-5-(pyridine-4-yl)-3*H*-1,3,4-oxadiazole-2-one of type (9) and studied their antimycobacterial activity. Krishna Kant Jha et al.[48] have reported antimicrobial activity of oxadiazole derivatives.



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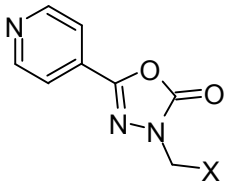
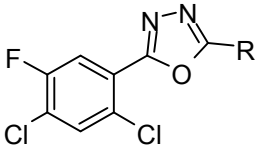


(9)

Hagen-Heinrich Hennies and coresearchers[49] have patented some novel 2-pyrrolidin-2-yl-(1,3,4)-oxadiazole derivatives which have a pronounced anti-depressive and analgesic effect. J. A. Christopher et al.[50] have documented effect of 1,3,4-oxadiazoles on human immunodeficiency virus. S. J. Gilani et al.[51] have synthesized some oxadiazoles as anti-inflammatory and analgesic agents. K. Subrahmanya Bhat et

al.[52] have prepared new fluorine containing 1,3,4-oxadiazoles (10) and reported them as potential antibacterial and anticancer agents. T. P. Mohan et al.[53] have synthesized 2,5-disubstituted-1,3,4-oxadiazole derivatives (11) and screened for their insecticidal activity. Ronald Kim et al.[54] have discovered oxadiazole derivatives useful as protease inhibitors.



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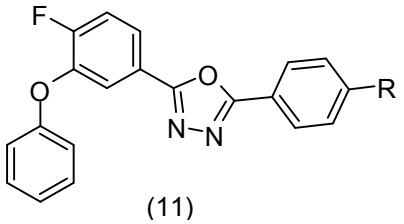
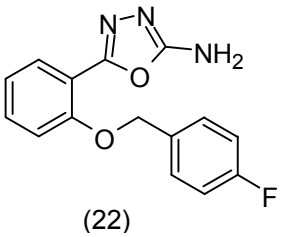


(11)

Mohd Amir and Kumar Shikha[55] have documented anti-inflammatory, analgesic and ulcerogenic activity of some newly synthesized oxadiazoles. A. Ali et al.[56] have investigated some oxadiazole derivatives possessing antimicrobial and anti-HIV-1 activity. A. Sherif et al.[57] have reported oxadiazoles as potential antitumor and anti-HIV agents. A. Zarghi et al.[58] have synthesized 2-amino-5-(2-benzyloxyphenyl)-1,3,4-oxadiazoles (12) possessing anticonvulsant activity. M. Tareq et al.[59] have synthesized 2,5-disubstituted-1,3,4-oxadiazoles (13) useful as tyrosinase inhibitors.



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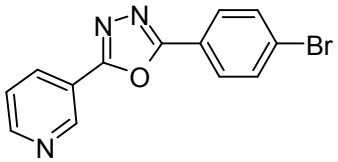
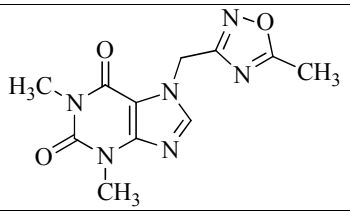
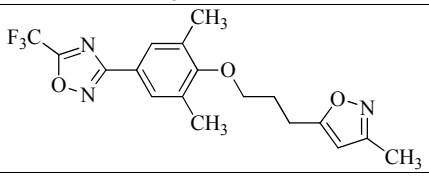
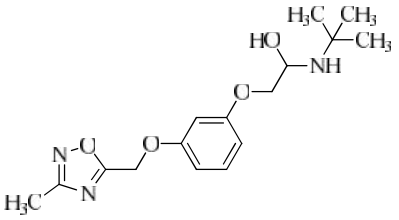
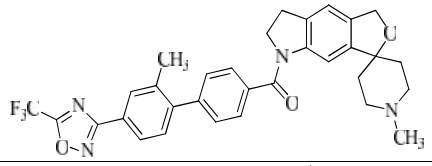
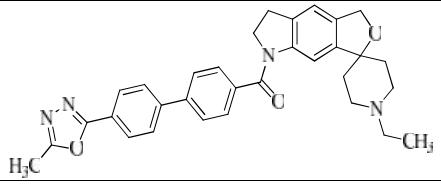
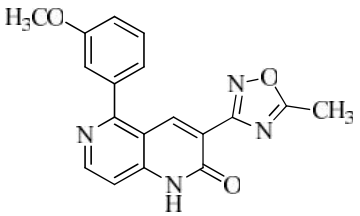
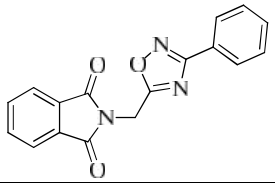
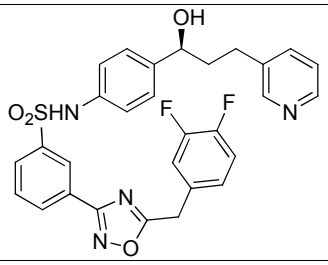
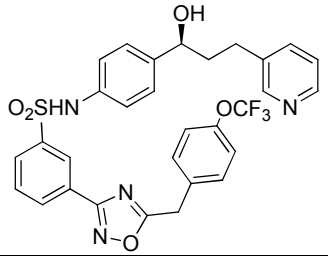
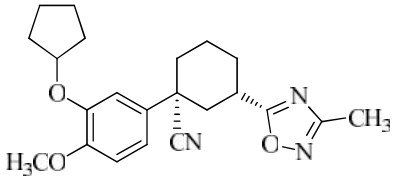
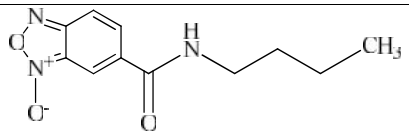
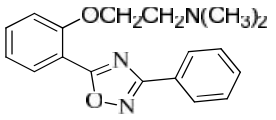


(23)

Example of some drugs & derivatives (containing oxadiazole scaffold) under Preclinical / clinical trials.

Sr. No	Chemical structure	Activity	Phase	Originator
1		Antitussive, Bronchodilator	Phase-I	Sanofi-Synthlabo
2		Antirhinoviral, Antiviral	Phase-III	Viro pharma
3		Antihypertensive, Antianginal, Antiglaucoma agent, Beta-adrenoceptor antagonist	Phase-II	Center for Chemistry of Drugs
4		Antidepressants, Anxiolytic, 5-HT1D Antagonist	Biological testing	Smithkline Beecham
5		Antidepressants, Anxiolytic, 5-HT1D Inverse agonist	Preclinical	Smithkline Beecham
6		Cognition enhancing drug, GABA(A) receptor modulator, GABA(A) B2 site inverse agonist	Preclinical	Dainoppon pharma
7		Analgesic	Preclinical	Universidade federal pernambuco

Sr. No	Chemical structure	Activity	Phase	Originator
8		Antiobesity drug, Antidiabetic drug, Beta3 adrenoce tor agonist	Preclinical	Merck
9		Antiobesity drug, Antidiabetic drug, Beta3 adrenoceptor agonist	Preclinical	Merck
10		Bronchodilator, Phosphodiesterase Inhibitor	Preclinical	Smithkline Beecham
11		Antitrypanosomal	Preclinical	Universidad delarepublica
12		Antiepileptic drug,Neuronal Injury Inhibitor, Sodium channel blocker	Preclinical	Boehringer Ingelaeim

In view of getting enhanced biological activities showed by 1,3,4-oxadiazoles, to prompted us to synthesize 1,3,4-oxadiazole derivatives, which have been described as under.

SECTION-I: SYNTHESIS AND BIOLOGICAL SCREENING OF 5-ARYL-2-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-1,3,4-OXADIAZOLE

SECTION-II: SYNTHESIS AND BIOLOGICAL SCREENING OF 5-ARYL-2-(6-FLUORO-3,4-DIHYDRO-2H-CHROMEN-2-YL)-1,3,4-OXADIAZOLE

SECTION-I

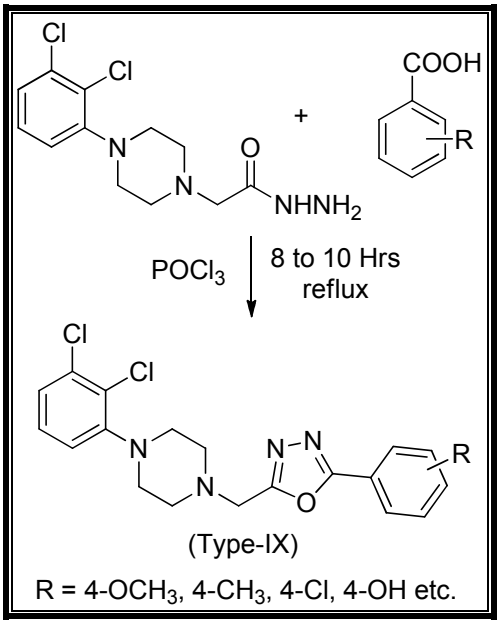
SYNTHESIS AND BIOLOGICAL SCREENING OF 5-ARYL-2-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-1,3,4-OXADIAZOLE

A large number of heterocycles bearing oxadiazole moiety have been reported for various biological activities. Oxadiazole derivatives have been drawn the attention of chemist due to their diversified biological activities. In view of these facts, the synthesis of some novel 2,5-disubstituted-1,3,4-oxadiazole derivative was undertaken. Oxadiazoles of type (IX) have been prepared by condensation of *2*-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide with different aromatic acid in phosphorous oxychloride.

The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

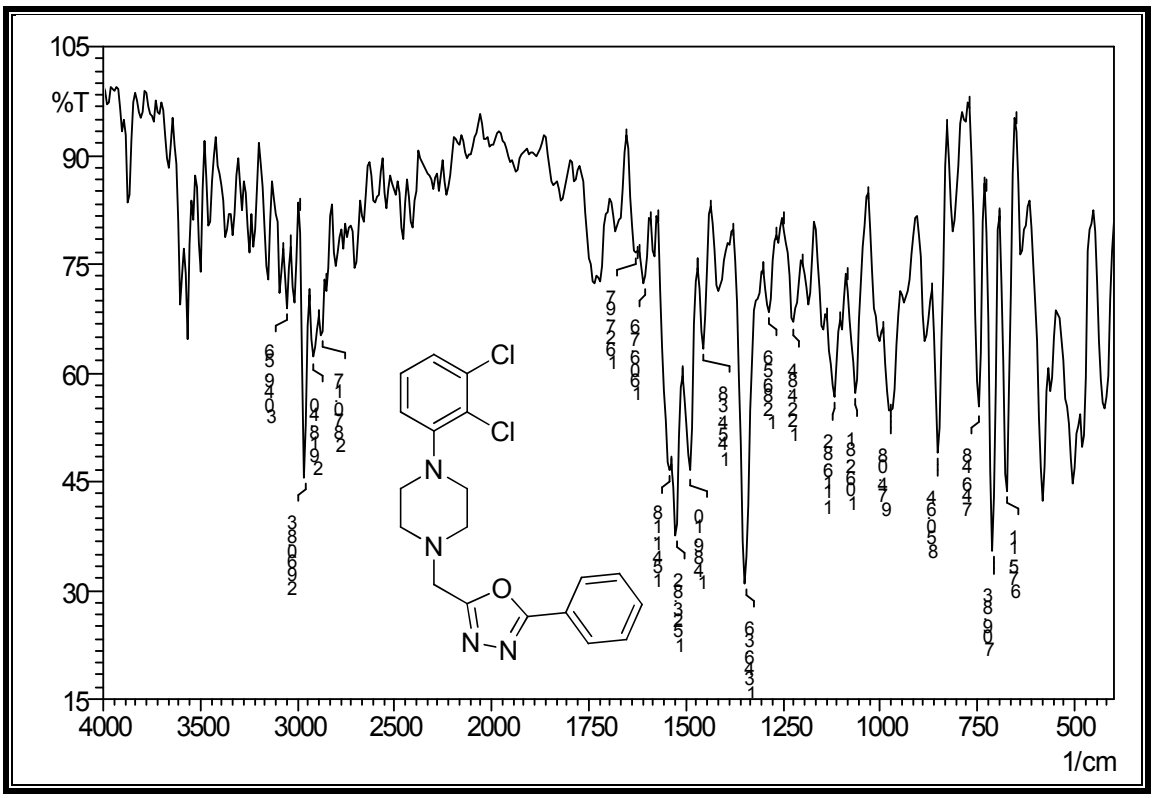
REACTION SCHEME



(Type-IX)

R = 4-OCH_3, 4-CH_3, 4-Cl, 4-OH etc.

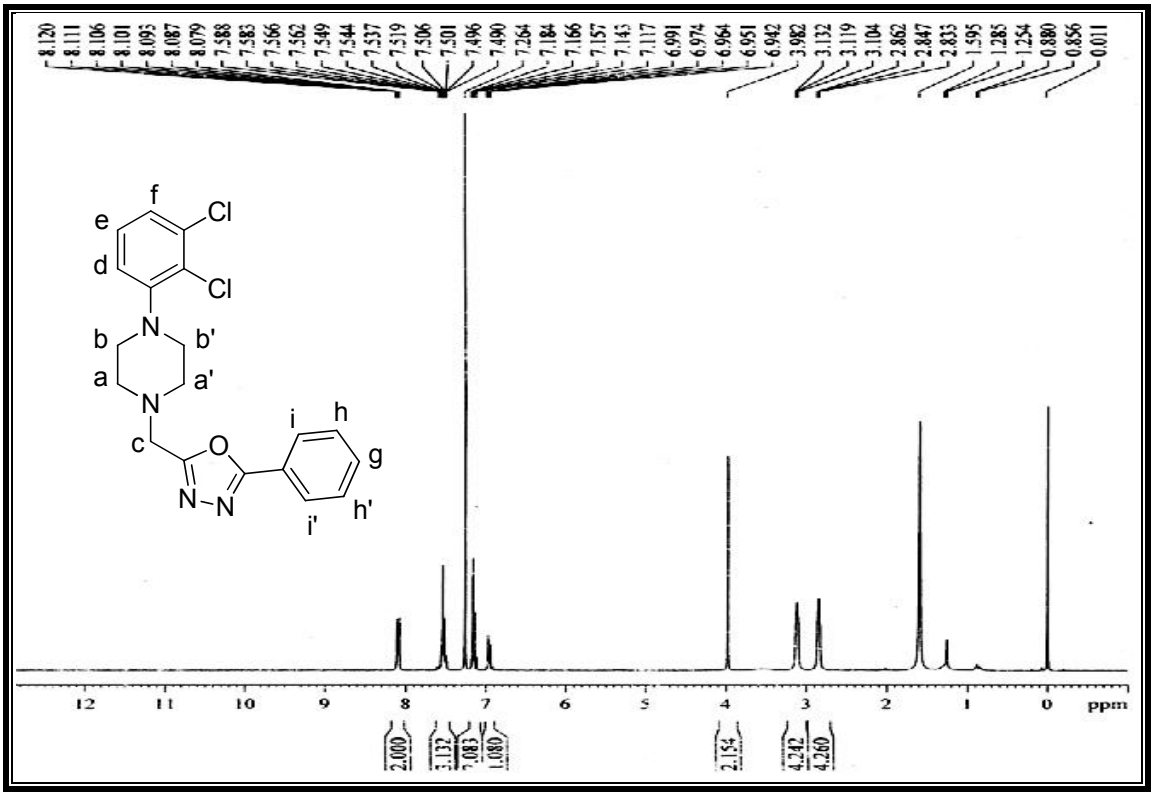
IR SPECTRUM OF 2-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-5-PHENYL-1,3,4-OXADIAZOLE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2960	2975-2920	60
	C-H str. (sym.)	2870	2880-2860	”
	C-H def. (asym.)	1454	1470-1435	”
	C-H def. (sym.)	1346	1395-1370	”
Aromatic	C-H str.	3049	3100-3000	”
	C=C str.	1523	1585-1480	”
	C-H i.p. def.	1116	1125-1090	”
	C-H o.o.p. def.	850	860-810	”
Oxadiazole	C=N str.	1606	1650-1580	61
	N-N str.	1224	1220-1020	”
	-C-O-C- str.	1062	1075-1020	”
Halide	C-Cl str.	850	850-650	62

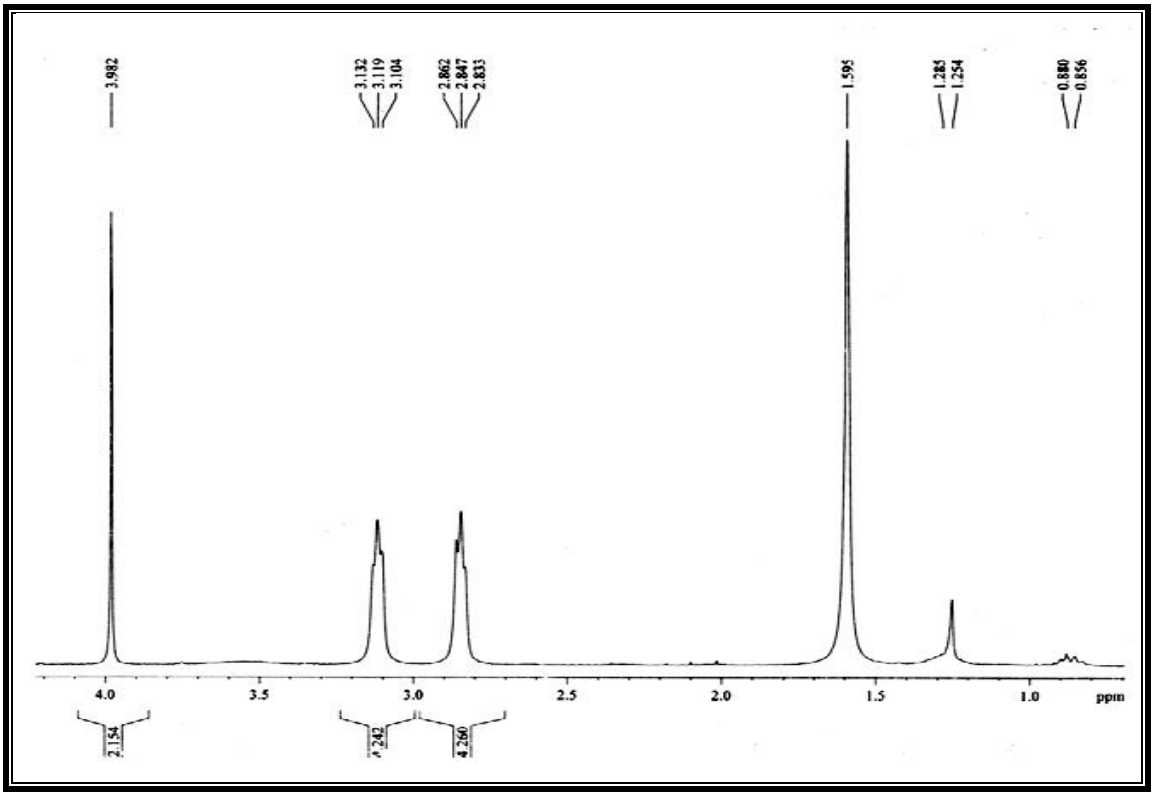
^{1}H-NMR SPECTRUM OF 2-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-5-PHENYL-1,3,4-OXADIAZOLE

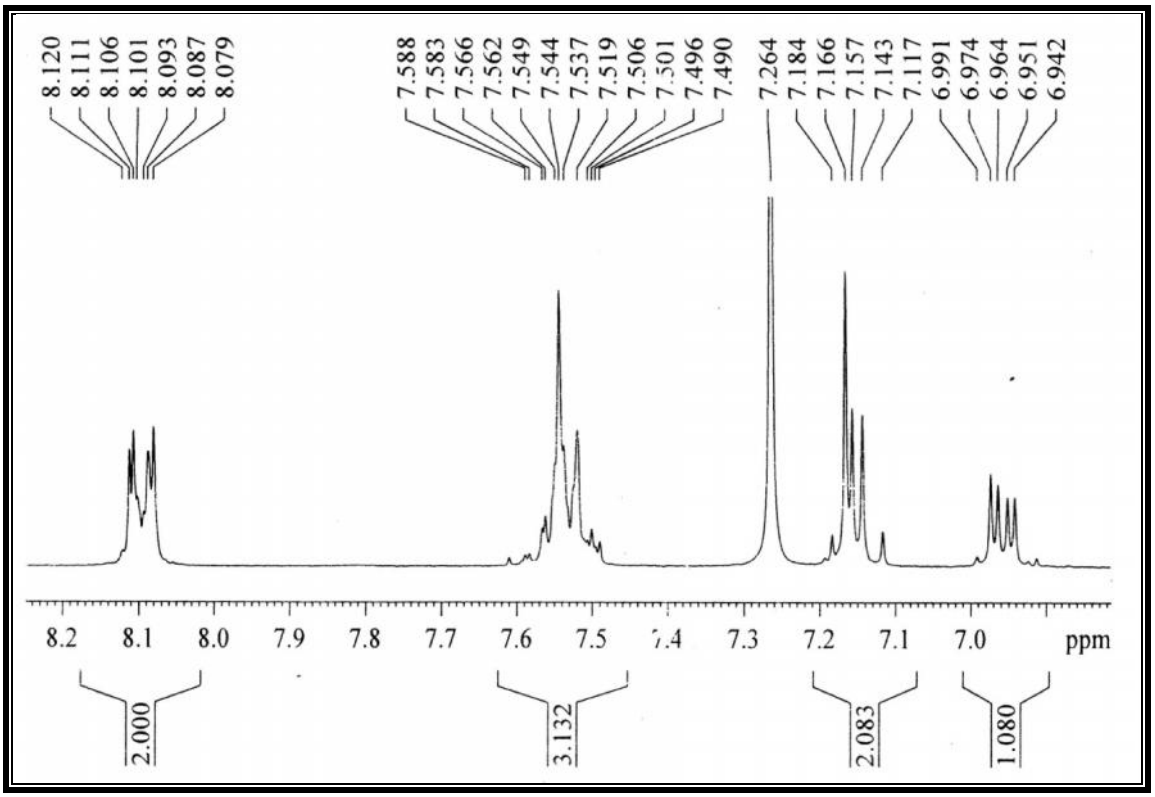


Internal Standard: TMS; Solvent: $CDCl_3$ Instrument: BRUKER Spectrometer (300MHz)

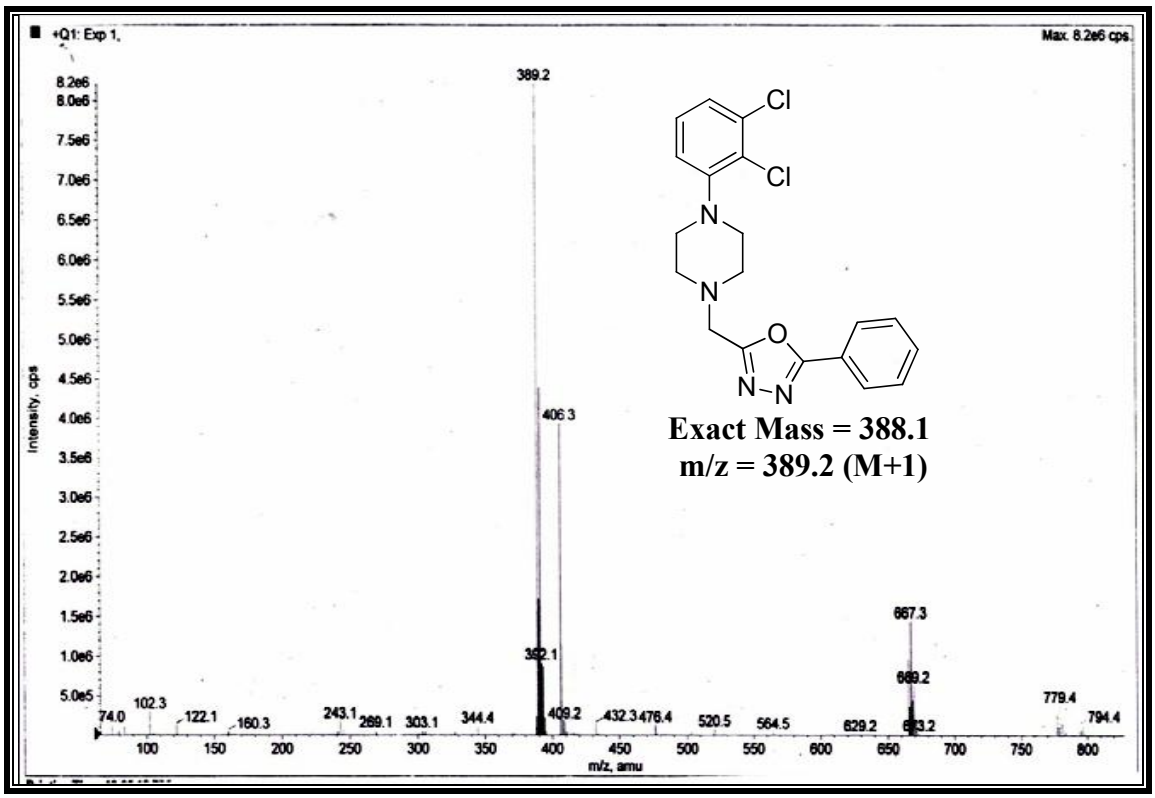
Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	2.83-2.86	4H	triplet	-C<u>H</u>$_2$ (a,a')	-
2	3.10-3.13	4H	triplet	-C<u>H</u>$_2$ (b,b')	-
3	3.98	2H	singlet	-C<u>H</u>$_2$ (c)	-
4	6.94-6.97	1H	double doublet	Ar-H (d)	2.7 & 6.6
5	7.11-7.18	2H	multiplet	Ar-H (e,f)	-
6	7.49-7.58	3H	multiplet	Ar-H (g,h,h')	-
7	8.07-8.11	2H	double doublet	Ar-H (i,i')	2.4 & 8.1

EXPANDED ^{1}H-NMR SPECTRUM

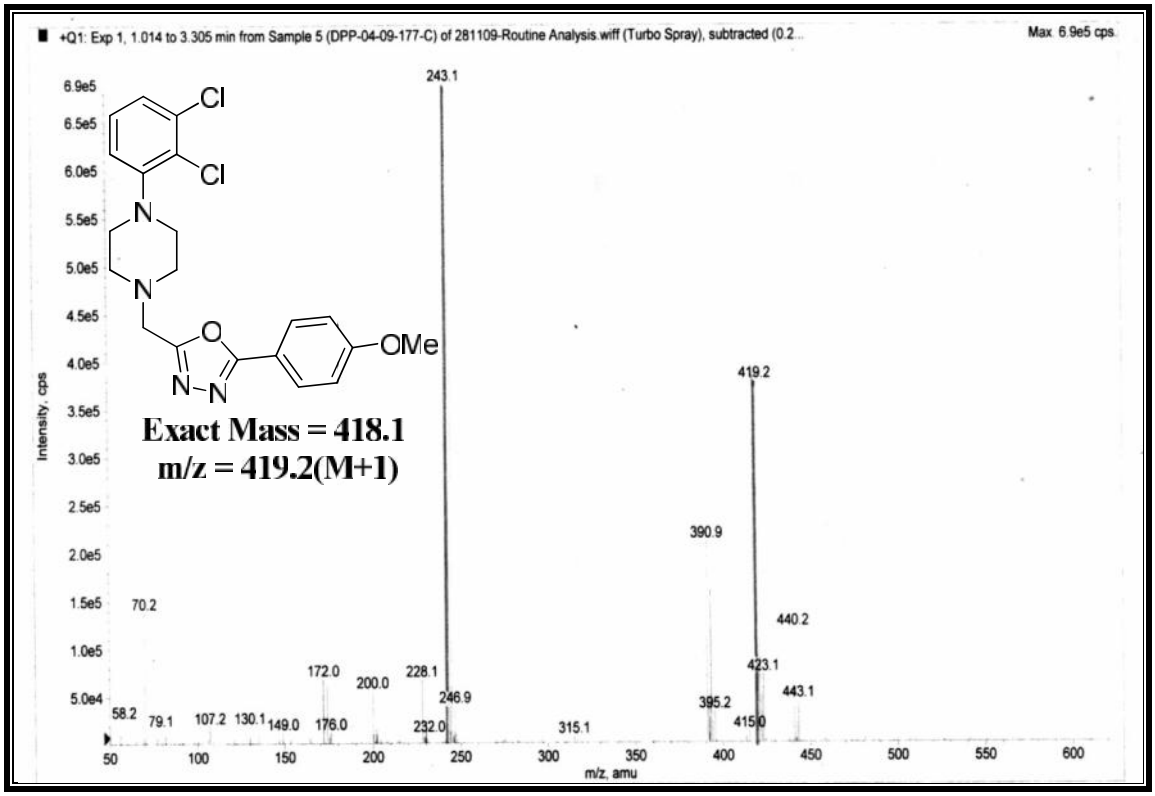




MASS SPECTRUM OF 2-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-5-PHENYL-1,3,4-OXADIAZOLE



MASS SPECTRUM OF 2-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-5-(4-METHOXYPHENYL)-1,3,4-OXADIAZOLE



EXPERIMENTAL

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. 40 % Ethyl acetate in hexane was used as a mobile phase.

[A] SYNTHESIS OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL) ACETOHYDRAZIDE

See, Chapter-2, Part-I, Section-I, Experimental [B], Page no. 72.

[B] SYNTHESIS OF 2-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL) METHYL)-5-PHENYL-1,3,4-OXADIAZOLE

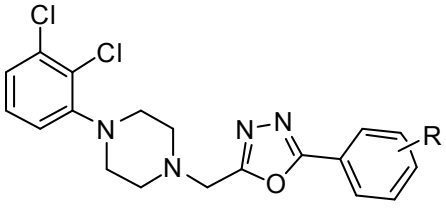
A mixture of 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide (1.51g, 0.005 mol) and benzoic acid (0.61g, 0.005 mol) in phosphorous oxychloride (5 ml) was refluxed for 8 hrs. The content was cooled, poured into crushed ice and neutralized with sodium bicarbonate solution. Obtained solid was filtered out, washed with water and dried. The crude product was purified by column chromatography on silica gel (60-120 mesh size) (Eluent = Ethyl acetate:hexane (3:7)) to obtain pure product. Yield 69 %, M. P. 192-194 °C ($C_{19}H_{18}Cl_2N_4O$; Required: C, 58.62; H, 4.66; N, 14.39 %; Found: C, 58.36; H, 4.58; N, 14.30 %).

Similarly other 1-(2,3-dichlorophenyl)-4-((5-aryl-1,3,4-oxadiazol-2-yl)methyl) piperazine **(9a-j)** were prepared. The physical constants are recorded in **Table-9a**, Page no. 190.

[C] BIOLOGICAL EVALUATION OF 5-ARYL-2-((4-(2,3- DICHLOROPHEN-YL)PIPERAZIN-1-YL)METHYL)-1,3,4-OXADIAZOLE

Antimicrobial testing was carried out as described in Chapter-1, Section-I, Experimental Section [C], Page no. 37. The results obtained from antimicrobial testing are recorded in **Table-9b**, Page no. 191

TABLE-9a: PHYSICAL CONSTANTS OF 5-ARYL-2-((4-(2,3-DICHLOROPHEN-YL)PIPERAZIN-1-YL)METHYL)-1,3,4-OXADIAZOLE



Sr. No.	Substitution R	Molecular Formula/ Molecular Weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
9a	H	$C_{19}H_{18}Cl_2N_4O$ 389.28	192-194	69	58.62 58.36	4.66 4.58	14.39 14.30
9b	4-OCH_3	$C_{20}H_{20}Cl_2N_4O_2$ 419.30	205-208	76	57.29 57.18	4.81 4.77	13.36 13.22
9c	4-CH_3	$C_{20}H_{20}Cl_2N_4O$ 403.30	168-171	70	59.56 59.41	5.00 4.86	13.89 13.72
9d	4-NO_2	$C_{19}H_{17}Cl_2N_5O_3$ 434.28	256-257	62	52.55 52.36	3.95 3.91	16.13 15.96
9e	4-F	$C_{19}H_{17}Cl_3N_4O$ 407.27	230-233	68	53.06 52.78	4.21 4.06	13.76 13.51
9f	4-Cl	$C_{19}H_{17}Cl_3N_4O$ 423.72	221-222	75	53.86 53.58	4.04 3.89	13.22 13.04
9g	4-Br	$C_{19}H_{17}BrCl_2N_4O$ 468.17	233-236	71	48.74 48.52	3.66 3.78	11.97 11.84
9h	4-OH	$C_{19}H_{18}Cl_2N_4O_2$ 405.28	231-233	54	56.31 56.09	4.48 4.33	13.82 13.71
9i	3- CH_3	$C_{20}H_{20}Cl_2N_4O$ 403.30	185-188	68	59.56 59.39	5.00 4.88	13.89 13.80
9j	2-NO_2-5-Cl	$C_{19}H_{16}Cl_3N_5O_3$ 468.72	244-247	62	48.69 48.57	3.44 3.38	14.94 14.83

TABLE-9b: BIOLOGICAL SCREENING OF 5-ARYL-2-((4-(2,3-DICHLOROPHEN-YL)PIPERAZIN-1-YL)METHYL)-1,3,4-OXADIAZOLE

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	9a	500	500	200	200	1000	250	500
2	9b	250	100	500	100	**250**	500	500
3	9c	250	500	100	250	500	500	500
4	9d	500	250	200	200	1000	1000	>1000
5	9e	**100**	500	200	250	500	200	250
6	9f	500	250	**62.5**	100	**200**	1000	>1000
7	9g	250	500	200	100	500	1000	>1000
8	9h	200	250	500	250	500	>1000	>1000
9	9i	500	250	250	500	500	500	1000
10	9j	250	100	250	250	1000	250	500

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*/S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted oxadiazole **9e** (R= 4-F) against *S.aureus* and **9f** (R= 4-Cl) against *E-coli* possess excellent activity compared to ampicillin. While **9h** (R= 4-OH) against *S.aureus*, **9b** (R= 4-OMe) & **9j** (R= 2-NO_2-5-Cl) against *S.pyogenus,* **9c** (R= 4-Me) against *E-coli* and **9b** (R= 4-OMe) & **9f** (R= 4-Cl) against *P.aeruginos,* exhibit moderate activity as compared to ampicillin. The remaining compounds display moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted oxadiazoles **9b** (R= 4-OMe) & **9f** (R= 4-Cl) demonstrate highly promissing activity against *C.albicans* as compared to greseofulvin. While **9a** (R= -H), **9e** (R= 4-F) & **9j** (R= 2-NO_2-5-Cl) against *A.niger* and **9e** (R= 4-F) against *A.*clavatus, possess moderate activity as compare to greseofulvin. The remaining compounds show moderate to poor activity against all three bacterial species.

SECTION-II

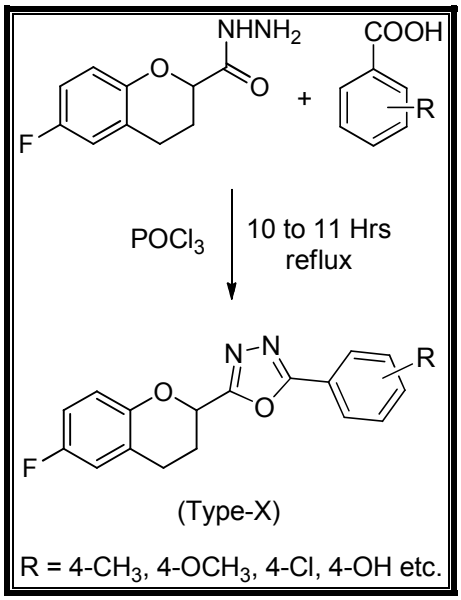
SYNTHESIS AND BIOLOGICAL SCREENING OF 5-ARYL-2-(6-FLUORO-3,4-DIHYDRO-2*H*-CHROMEN-2-YL)- 1,3,4-OXADIAZOLE

Synthesis of 1,3,4-oxadiazole derivatives has attracted considerable attention in view of therapeutic applications. A large number of heterocycles bearing oxadiazole moiety have been reported for various biological activities. Looking to this, the synthesis of 1,3,4-oxadiazoles was undertaken. Oxadiazoles of type (X) have been prepared by condensation of 6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide with different aromatic acids in phosphorous oxychloride.

The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

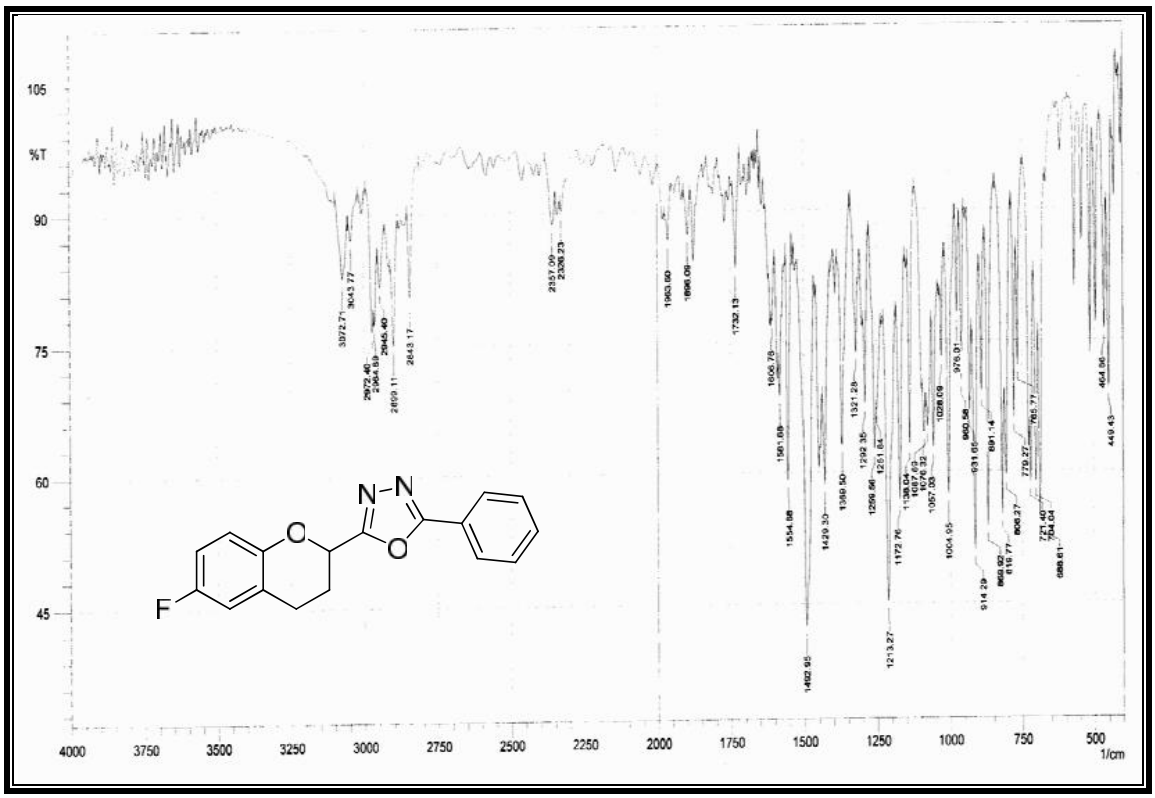
REACTION SCHEME



(Type-X)

R = 4-CH_3, 4-OCH_3, 4-Cl, 4-OH etc.

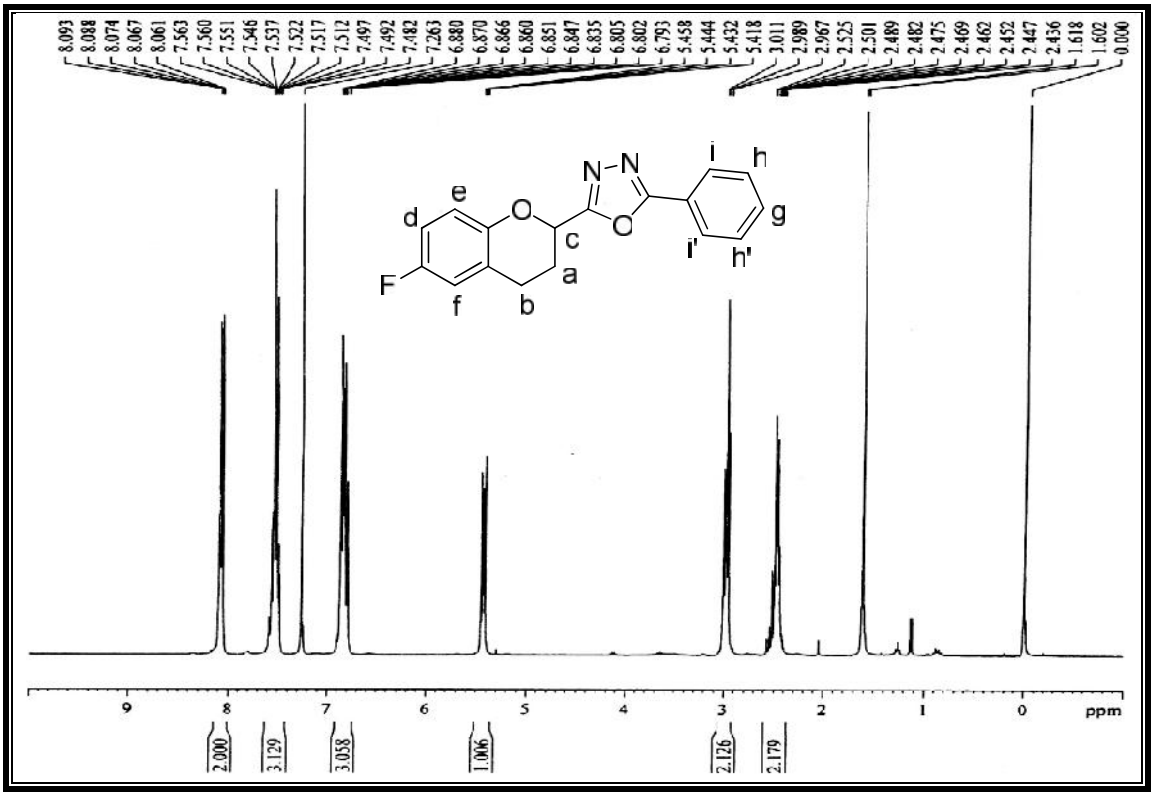
IR SPECTRUM OF 2-(6-FLUORO-3,4-DIHYDRO-2*H*-CHROMEN-2-YL)-5-PHENYL-1,3,4-OXADIAZOLE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2972	2975-2920	60
	C-H str. (sym.)	2843	2880-2860	”
	C-H def. (asym.)	1429	1470-1435	”
	C-H def. (sym.)	1369	1395-1370	”
Aromatic	C-H str.	3072	3100-3000	”
	C=C str.	1492	1585-1480	”
	C-H i.p. def.	1087	1125-1090	”
	C-H o.o.p. def.	819	860-810	”
Oxadiazole	C=N str.	1581	1650-1580	61
	N-N str.	1172	1220-1020	”
	-C-O-C- str.	1057	1075-1020	”
Halide	C-F str.	819	850-650	62

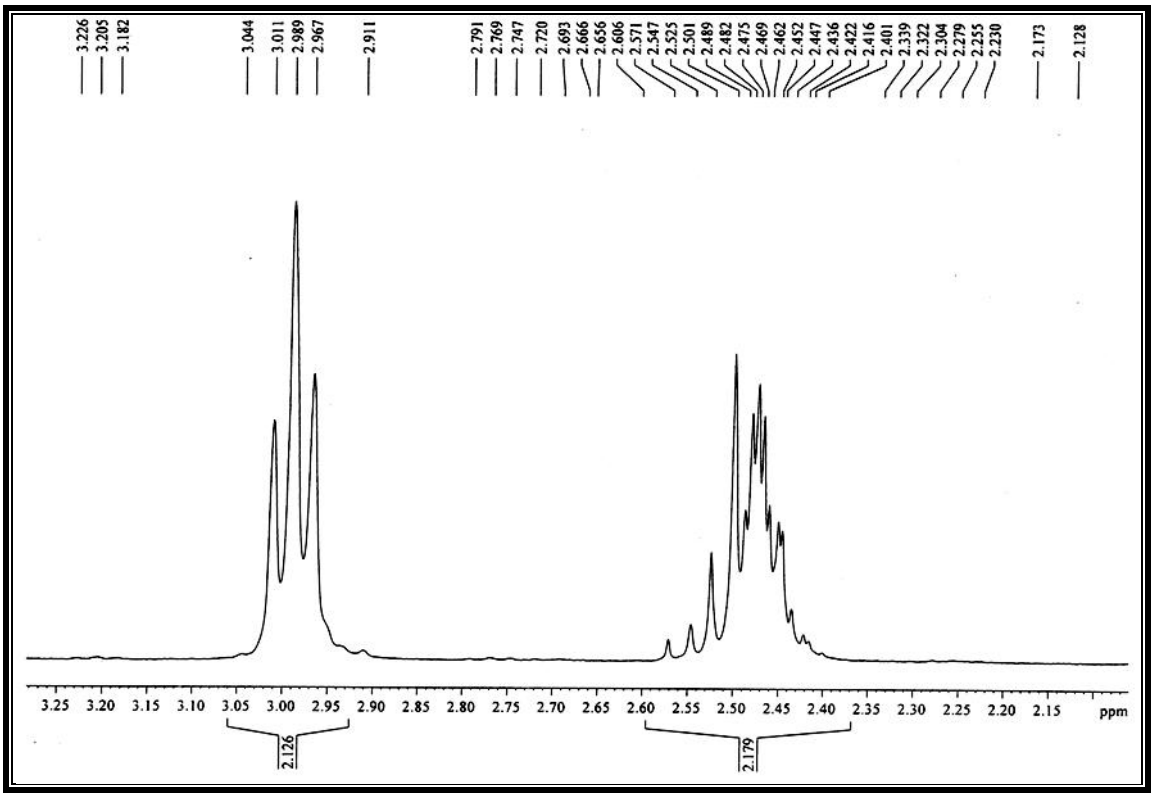
^{1}H-NMR SPECTRUM OF 2-(6-FLUORO-3,4-DIHYDRO-2*H*-CHROMEN-2-YL)-5-PHENYL-1,3,4-OXADIAZOLE

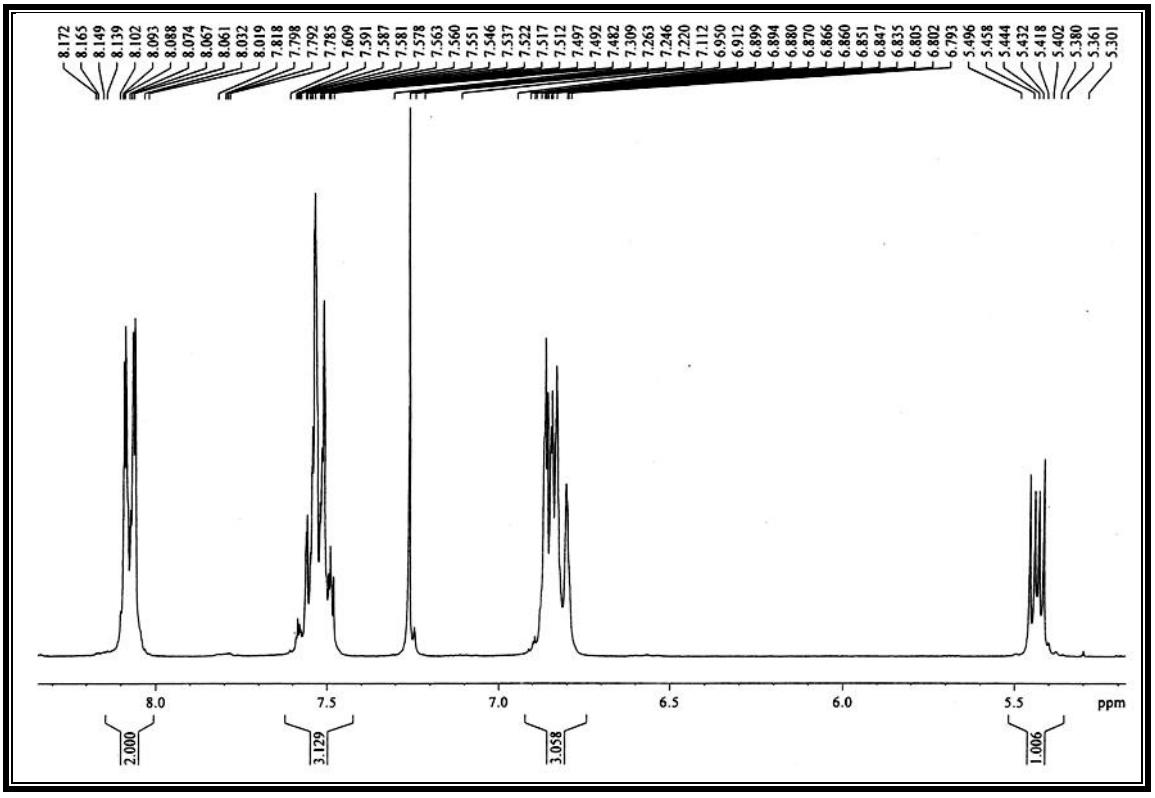


Internal Standard: TMS; Solvent: $CDCl_3$ Instrument: BRUKER Spectrometer (300MHz)

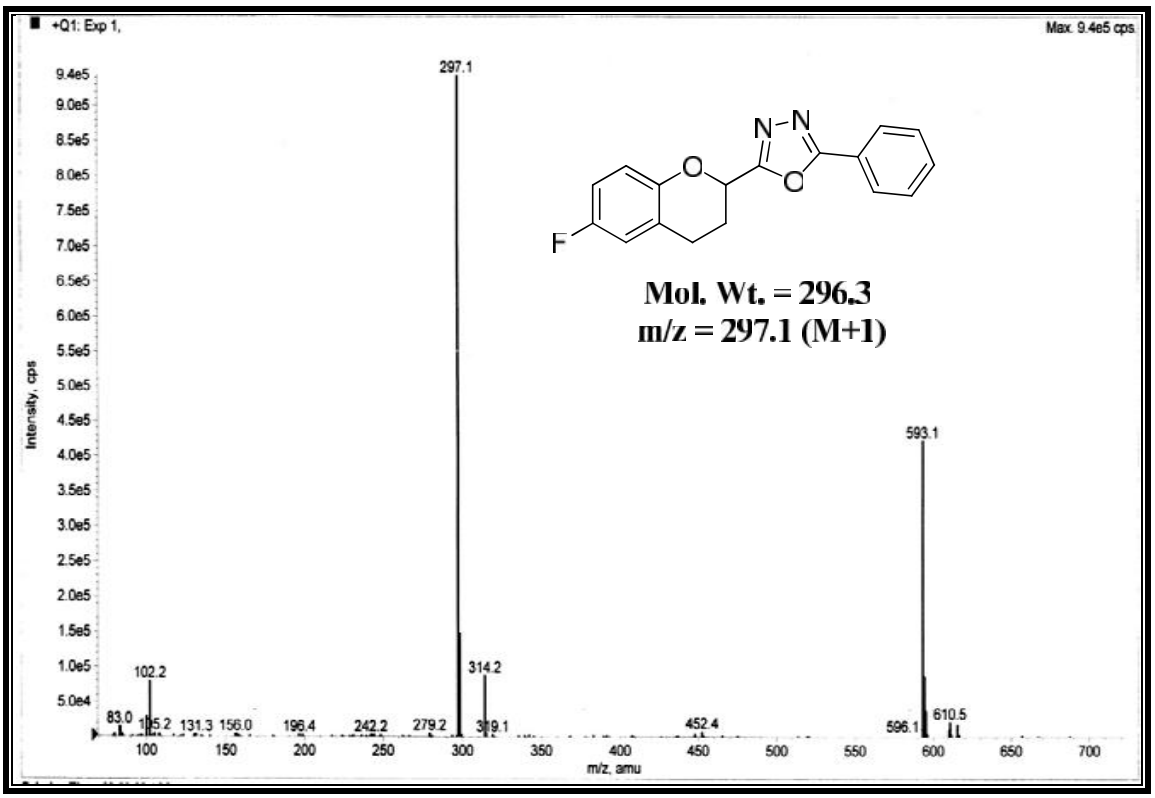
Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	2.43-2.52	2H	multiplet	-C$\underline{H}_2$ (a)	-
2	2.96-3.01	2H	triplet	-C$\underline{H}_2$ (b)	-
3	5.41-5.45	1H	double doublet	-C$\underline{H}$ (c)	4.2 & 7.8
4	6.79-6.88	3H	multiplet	Ar-H (d,e,f)	-
5	7.49-7.56	3H	multiplet	Ar-H (g,h,h')	-
6	8.06-8.09	2H	multiplet	Ar-H (i,i')	-

EXPANDED ^{1}H-NMR SPECTRUM

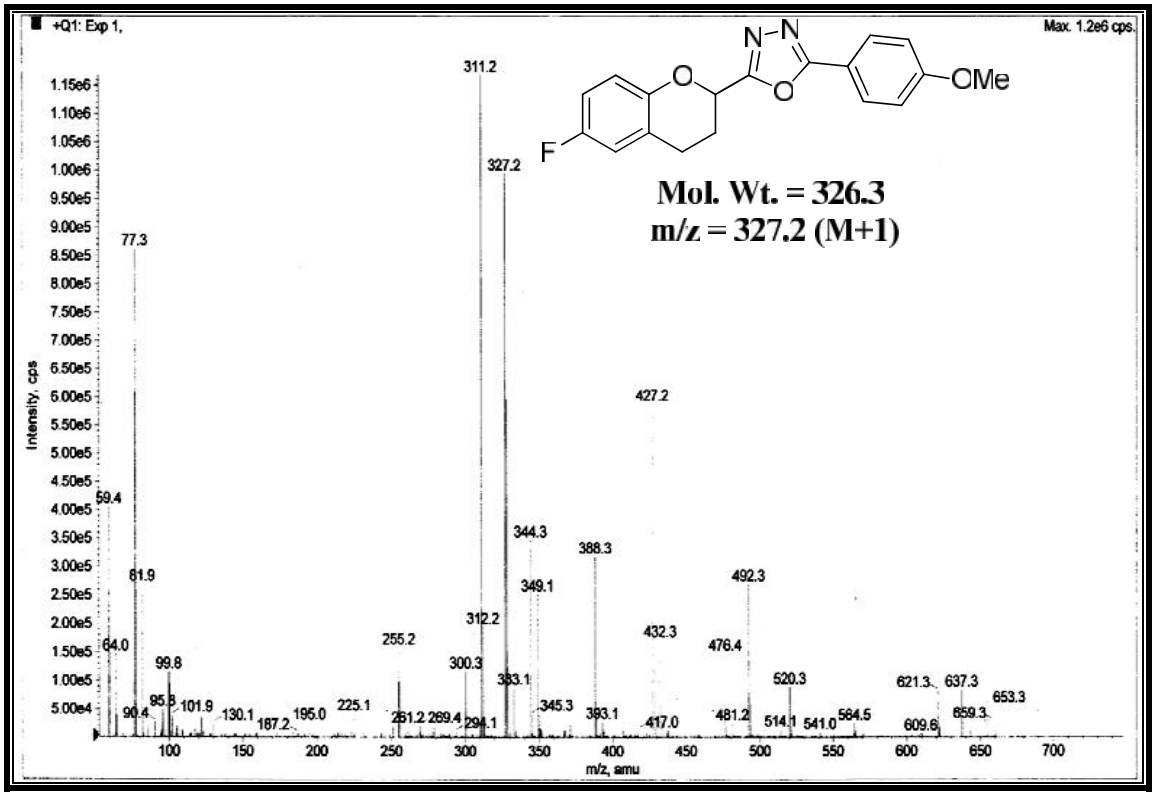




MASS SPECTRUM OF 2-(6-FLUORO-3,4-DIHYDRO-2*H*-CHROMEN-2-YL)-5-PHENYL-1,3,4-OXADIAZOLE



MASS SPECTRUM OF 2-(6-FLUORO-3,4-DIHYDRO-2*H*-CHROMEN-2-YL)-5-(4-METHOXYPHENYL)-1,3,4-OXADIAZOLE



EXPERIMENTAL

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. 30 % Ethyl acetate in hexane was used as a mobile phase.

[A] SYNTHESIS OF OF 6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

See, Chapter-2, Part-II, Section-I, Experimental [B], Page no. 93.

[B] SYNTHESIS OF 2-(6-FLUORO-3,4-DIHYDRO-2*H*-CHROMEN-2-YL)-5-PHENYL-1,3,4-OXADIAZOLE

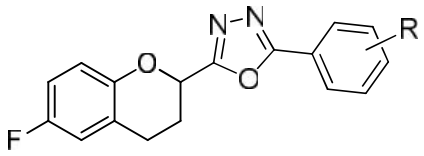
A mixture of 6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide (1.05g, 0.005 mol) and benzoic acid (0.61g, 0.005 mol) in phosphorous oxychloride (5 ml) was refluxed for 10 hrs. The content was cooled, poured into crushed ice and neutralized with sodium bicarbonate solution. Obtained solid was filtered out, washed with water and dried. The crude product was purified by column chromatography on silica gel (60-120 mesh size) (Eluent = Ethyl acetate:hexane (2:8)) to obtain pure product. Yield: 74 %**,** M. P. 153-156 °C ($C_{17}H_{13}FN_2O_2$; C, 68.91; H, 4.42; N, 9.45 %; Found: C, 68.70; H, 4.33; N, 9.39 %).

Similarly other 1-(2,3-dichlorophenyl)-4-((5-aryl-1,3,4-oxadiazol-2-yl)methyl) piperazine **(10a-j)** were prepared. The physical constants are recorded in **Table-10a**, Page no. 199.

[C] BIOLOGICAL EVALUATION OF 5-ARYL-2-(6-FLUORO-3,4-DIHYDRO-2*H*-CHROMEN-2-YL)- 1,3,4-OXADIAZOLE

Antimicrobial testing was carried out as described in Chapter-1**,** Section-I, Experimental Section-[C], Page no. 37. The results obtained from antimicrobial testing are recorded in **Table-10b**, Page no. 200.

TABLE-10a: PHYSICAL CONSTANTS OF 5-ARYL-2-(6-FLUORO-3,4-DIHYDRO-2*H*-CHROMEN-2-YL)- 1,3,4-OXADIAZOLE



Sr. No.	Substitution R	Molecular Formula/ Molecular Weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
10a	H	$C_{17}H_{13}FN_2O_2$ 296.30	153-156	74	68.91 68.70	4.42 4.33	9.45 9.39
10b	4-OCH_3	$C_{18}H_{15}FN_2O_3$ 326.32	129-131	67	66.25 66.09	4.63 4.51	8.58 8.51
10c	4-CH_3	$C_{18}H_{15}FN_2O_2$ 310.32	118-120	70	69.67 69.52	4.87 4.81	9.03 8.92
10d	4-NO_2	$C_{17}H_{12}FN_3O_4$ 341.29	171-172	59	59.83 59.69	3.54 3.48	12.31 12.24
10e	4-F	$C_{17}H_{12}F_2N_2O_2$ 314.29	146-149	65	64.97 64.68	3.85 3.74	8.91 8.80
10f	4-Cl	$C_{17}H_{12}ClFN_2O_2$ 330.74	104-105	73	61.73 61.51	3.66 3.58	8.47 8.41
10g	4-Br	$C_{17}H_{12}BrFN_2O_2$ 375.19	118-121	68	54.42 54.32	3.22 3.18	7.47 7.41
10h	4-OH	$C_{17}H_{13}FN_2O_3$ 312.30	169-172	52	65.38 65.24	4.20 4.14	8.97 8.89
10i	3- CH_3	$C_{18}H_{15}FN_2O_2$ 310.32	136-138	66	69.67 69.56	4.87 4.85	9.03 9.00
10j	2-NO_2-5-Cl	$C_{17}H_{11}ClFN_3O_4$ 375.74	166-168	56	54.34 54.25	2.95 2.91	11.18 11.11

TABLE-10b: BIOLOGICAL SCREENING OF 5-ARYL-2-(6-FLUORO-3,4-DIHYDRO-2*H*-CHROMEN-2-YL)- 1,3,4-OXADIAZOLE

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	10a	100	200	500	250	1000	250	500
2	10b	250	250	**50**	250	1000	>1000	>1000
3	10c	250	500	200	200	500	500	500
4	10d	500	250	250	200	500	1000	>1000
5	10e	**200**	100	250	100	**250**	500	500
6	10f	500	200	100	200	500	1000	1000
7	10g	500	500	250	500	500	>1000	>1000
8	10h	500	500	500	250	1000	200	200
9	10i	500	500	250	500	500	1000	1000
10	10j	100	200	200	200	500	>1000	>1000

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted oxadiazole **10e** (R= 4-F) against *S.aureus* and **10b** (R= 4-OMe) against *E-coli* display very good activity compared to ampicillin. While **10b** (R= 4-OMe) & **10c** (R= 4-Me) against *S.aureus*, **10e** (R= 4-F) against *S.pyogenus,* **10f** (R= 4-Cl) against *E-coli* and **10e** (R= 4-F) against *P.aeruginos,* possess moderate activity as compared to ampicillin. The remaining compounds exhibite moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted oxadiazoles **10e** (R= 4-F) exhibit excellent activity against *C.albicans* as compared to greseofulvin. While **10a** (R= -H) & **10h** (R= 4-OH) against *A.niger* and **10h** (R= 4-OH) against *A.clavatus,* show moderate activity as compare to greseofulvin. The remaining compounds display moderate to poor activity against all three bacterial species.

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Chapter-6

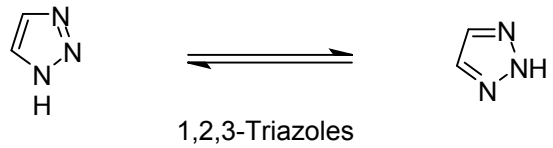
STUDIES ON TRIAZOLE DERIVATIVES

INTRODUCTION

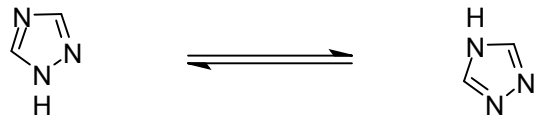
Triazoles are important class of heterocyclic compounds, found in many potent biologically active molecules.Triazole derivatives have occupied an important place in novel drug discovery process. Triazoles are well known five membered heterocyclic compounds and several procedures for their synthesis have been extensively studied. Such studies have been stimulated by various promising applications, especially in the case of nitrogen containing heterocyclic entities.

The knowledge of such applications has pointed out that nitrogen containing heterocycles are important target to be prepared for our research on biologically active heterocyclic analogous. Triazoles are of two types 1,2,3-triazole and 1,2,4-triazole .

Isomeric forms of triazole



1,2,3-Triazoles



1,2,4-Triazoles

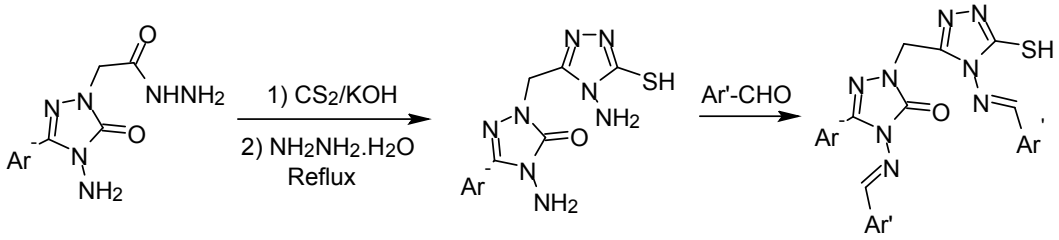
1,2,4-Triazoles represents a rapid developing field in morden heterocyclic chemistry. From literature it is predictable that, 1,2,4-triazoles represents important pharmacophores, and play a vital role as medicinal agents. A degree of respectability has been bestowed for 1,2,4-triazole derivatives due to their wide range of biological activities such as antifungal,[1] antitubercular[2] and anticancer.[3] Certain 1,2,4-triazoles also find applications in the preparation of photographic plates, polymers, and as analytical agents.[4]

Z. M. Hao[5] and S. T. Steven[6] have been studied briefly with the chemistry of 1,2,4-triazoles. Bladin[7,8] is a pioneer scientist in the field of triazole, who had synthesized the first derivative of 1,2,4-triazole in 1885. 1,2,4-Triazole derivatives not only known for their medicinal applications, but they are also used as analytical reagents[9], dyes and photographic chemicals[10] corrosion inhibitors[11,12] and in the preparation of polymers[13].

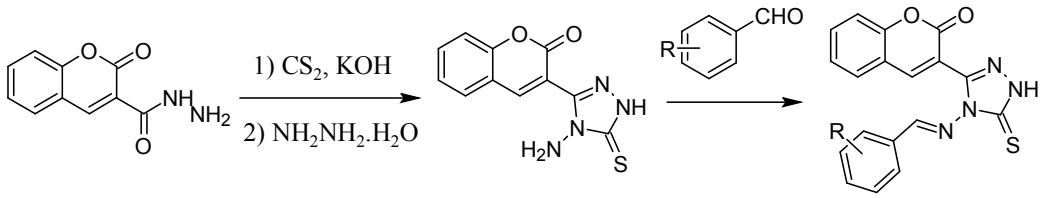
SYNTHETIC ASPECT

Several methods have been reported in the literature for the synthesis of 1,2,4-triazoles.

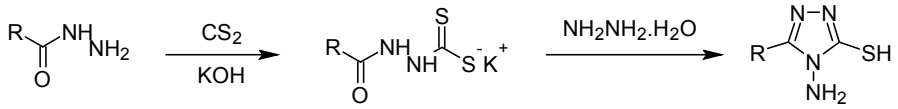
1. Ahmet Demirbas et al[14]. have synthesized 1,2,4-triazole derivative from the reaction of acetohydrazide dreivative with CS_2/KOH followed by hydrazine hydrate and aromatic aldehydes.



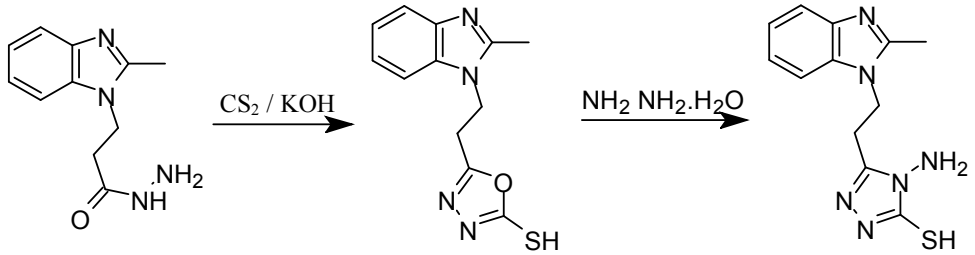
2. Mashooq A. Bhat et al[15]. Synthesized 3-(4-(((aryl)methylene)imino)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-2*H*-chromen-2-ones from 2-oxo-2*H*-cromene-3-carbohydrazide in 3 step.



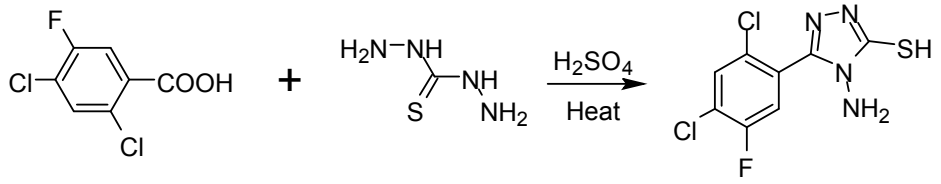
3. Reid and Heindel[16] reported that the reaction of aryl acid hydrazide with CS2/KOH and hydrazine hydrate yields triazoles.



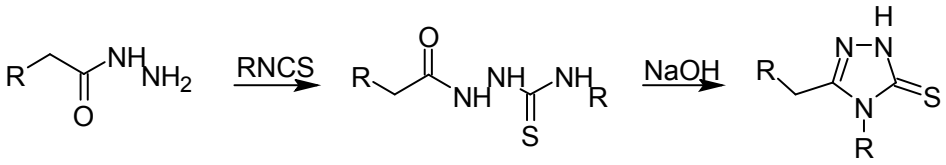
4. A .K. Mishra et al[17] have reported synthesis and antimicrobial activity of some triazole derivatives starting from 2-substituted-1*H*-benzimidazole.



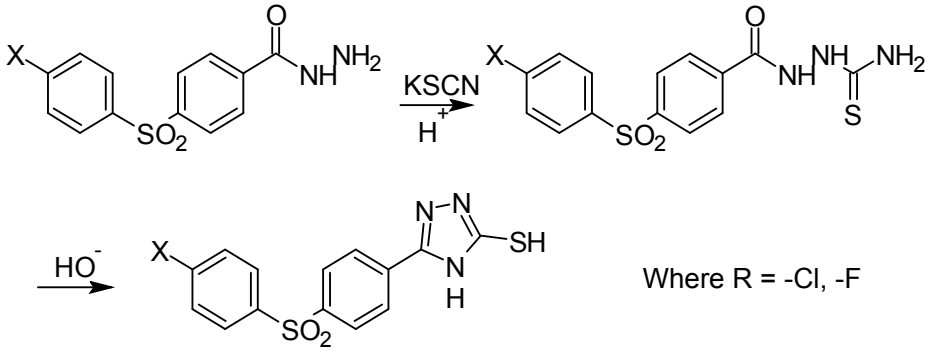
5. K. S. Bhat et al.[18] have synthesized 4-amino-3-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazol-5-thiol with the help of thiocarbohydrazide and 2,4-dichloro-5-fluoro benzoic acid.



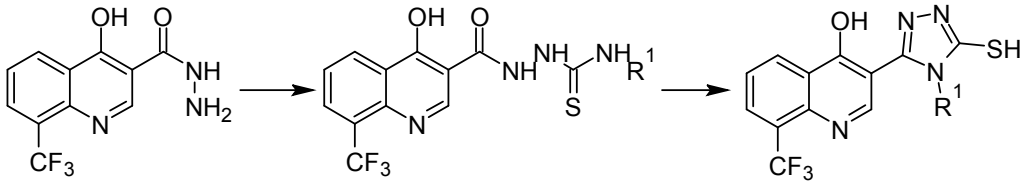
6. N. U. Guzeldemirci et al.[19] have prepared 1,2,4-triazoles in the presence of NaOH from Aryl acid hydrazide.



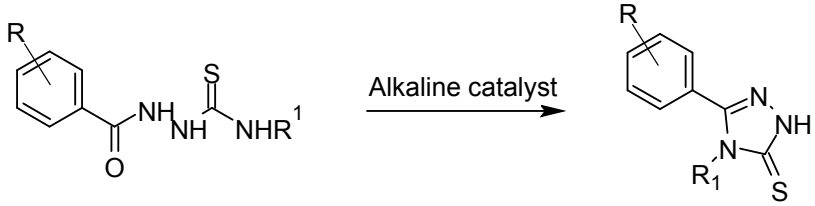
7. S. F. Barbuceanu et al.[20] have reported 5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thioles and It is prepared from 4-(4-X-phenylsulfonyl)-benzoicacid hydrazide.



8. Sumesh eswaran et al.[21] also synthesized triazole derivative by the reaction of 4-hydroxy-8-(trifluoromethyl)quinoline-3-carbohydrazide.

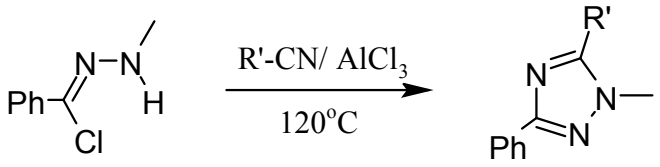


9. L. Labanauskas et al.[22] have prepared triazoles by the addition reaction of thiosemicarbazide with substituted benzoyl chloride in the presence of pyridine. Then the substituted thiosemicarbazide cyclised in water in the presence of alkaline catalyst.



10. K. Paulvannam et al[23] have developed an improved synthesis of 1,3,5-

trisubstituted 1,2,4-triazoles via Ag_2CO_3 mediated cyclization of triazenes. The reaction was complete within 3h and the products were isolated in moderate to high yields.

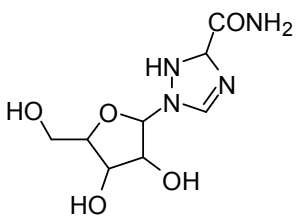


THERAPEUTIC IMPORTANCE

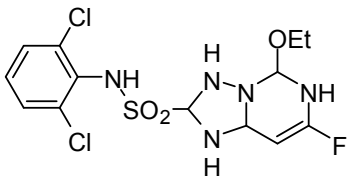
Triazoles are potential bioactive agents due to their wide spectrum of therapeutic importance. Literature survey reveals that various 1,2,4-triazole derivatives display significant biological activities. 3-Amino-1,2,4-triazole was the first 1,2,4-triazole to be manufactured on large scale from amino guanidine format, useful as herbicides[24], therapeutic activity of 1,2,4-triazoles are as under.

Bactericidal[25], Diuretic[26], Fungicidal[27], Herbicidal[28], Insecticidal and acaricidal[29], Plant growth regulator[30], Anticancer and Anti-HIV[31], Antileshmanial[32], Antitumor[33,34], Anti-depressant and anxiolytic[35], Antimicrobial[36], Antiviral[37], Antiinflammatory[38], Antihypertensive[39] and Anticonvulsant[40].

Hoong-Kun Fun et al.[41] have investigated 4-Amino-3-(1-naphthyloxymethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione. B. Kahveci et al.[42] have prepared 4-Arylmethyl-idene-amino-3-(R-benzyl)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones via microwave assisted synthesis which exhibited remarkable antifungal activity. Ram Janam Singh et al.[43] have synthesized 4-Aryl-5-(isomeric pyridoyl)-3*H*-1,2,4-triazoles as potent bacteriocidal agents which active against *S. aureus, E. coli, B. subtilis* and *P. aeruginosa*. Najim A. Al-Masoudi et al.[44] have suggested 5-Amino-4-phenyl-4*H*-1,2,4-triazole-3-thiol and their metal complexes which posses *vitro* anti-HIV activity. E. De Clercq et al.[45] screened ribavarin (11) for their antiviral and antimetabolic activities. Mckendry and co-workers[46] have synthesized triazole derivatives (12) and reported them as broad spectrum broadleaf herbicides.

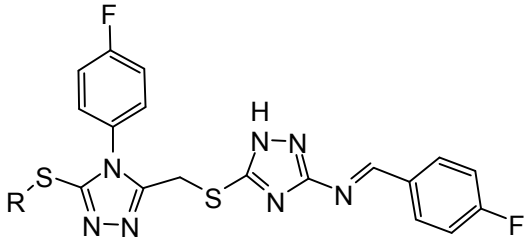


(11)



(12)

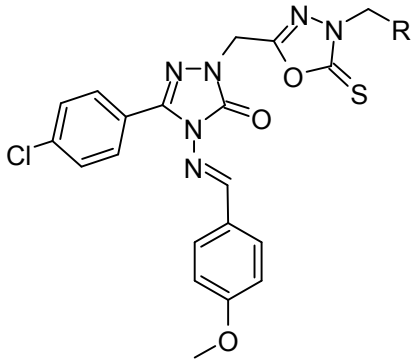
Sherin M. El-Feky et al[47] have synthesized a new series of 3,5-disubstituted triazoles (13) and evaluated for *invitro* antifungal and antibacterial activity. All the tested compounds showed significant antifungal activity against *micromycetes* compared to the commercial fungicide clotrimazole.



(13)

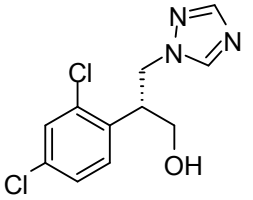
H. A. Abdel-Aziz et al.[48] have synthesized some piperidine based 1,3-thiazole, 1,3,4-thiadiazole, and 1,3-thiazolo[2,3-*c*]-1,2,4-triazole derivatives which posses anti-arrhythmic activity. B. F. Abdel-Wahab et al.[49] have reported 1,2,4-triazoles useful for antimicrobial agent.

Hakan Bekats et al[50] have synthesized some novel 2,4,5-trisubstituted-2,4-dihydro-3*H*-1,2,4-triazole-3-one (14) and all these compounds were screened for their antimicrobial activities and some of which were found to possess good or moderate activities against the test microorganisms.

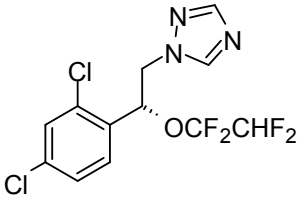


(14)

Daniele Binchi et al.[51] have screened pure stereoisomer of two triazole derivatives (15,16) for their antifungal activity against variety of fungi.



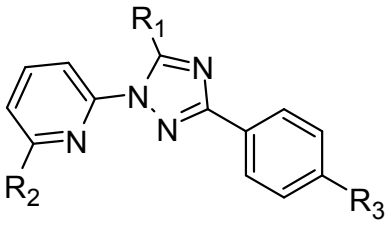
(15)



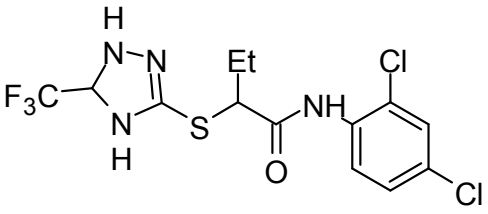
(16)

M. A. Kaldrikyan et al.[52] have discovered some benzofuryl-substituted-1,2,4-

triazoles and reported their antitumor activity. Dae-Kee Kim et al.[53] have been synthesized 1,2,4-triazole derivatives (17) and screened for their significant ALKS inhibitory activity. K. J. Fisher et al.[54] have synthesized 1,2,4-triazole derivatives (18) to study their pesticidal and herbicidal activity. Xiang-Shu Cui et al.[55] have formulated 3-substituted-4-(4-hexyloxyphenyl)-4*H*-1,2,4-triazoles as anticonvulsant agent. Maarouf et al.[56] have documented analgesic and anti-inflammatory activity of 1,2,4-triazole derivatives.



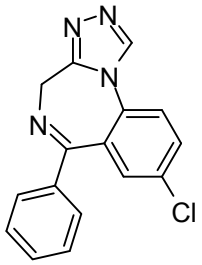
(17)



(18)

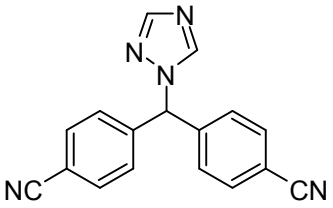
Drug molecules having 1,2,4-triazole nucleus with their activity are listed as under.

1. Estazoalm



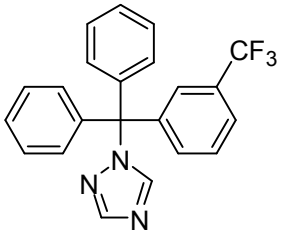
Receptor Agonist

2. Letrozole



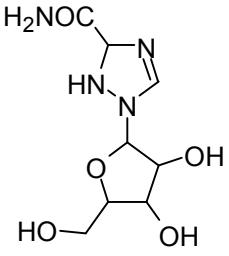
Antineoplastic

3. Fluotrimazole



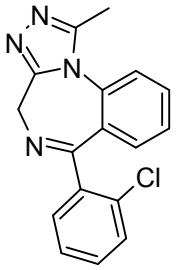
Fungicide

4. Ribavarin



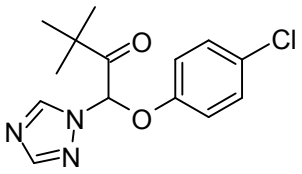
Antiviral, Antiinfection

5. Triazolam



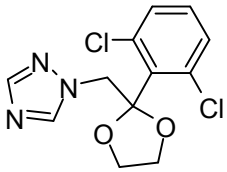
Plant growth regulator

6. Triadimenol



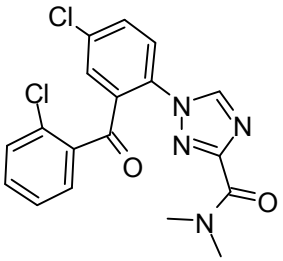
Fungicide

7. Azaconazole



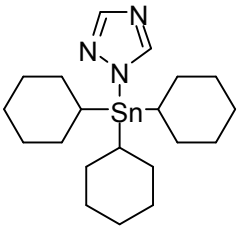
Antifungal

8. Rilmazafone



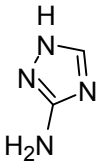
Sedative,hypnotic

9. Azocyclotin



Pesticide

10. Amitrole



Antithyroid activity

Thus the important role displayed by triazole moiety for various therapeutic and medicinal activities prompted us to synthesize some Schiff base derivative bearing triazole moiety, which have been described as under.

SECTION-I: SYNTHESIS AND BIOLOGICAL SCREENING OF 4-((ARYLMETHYLIDINE)AMINO)-5-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-4*H*-1,2,4-TRIAZOLE-3-THIOL

SECTION-I

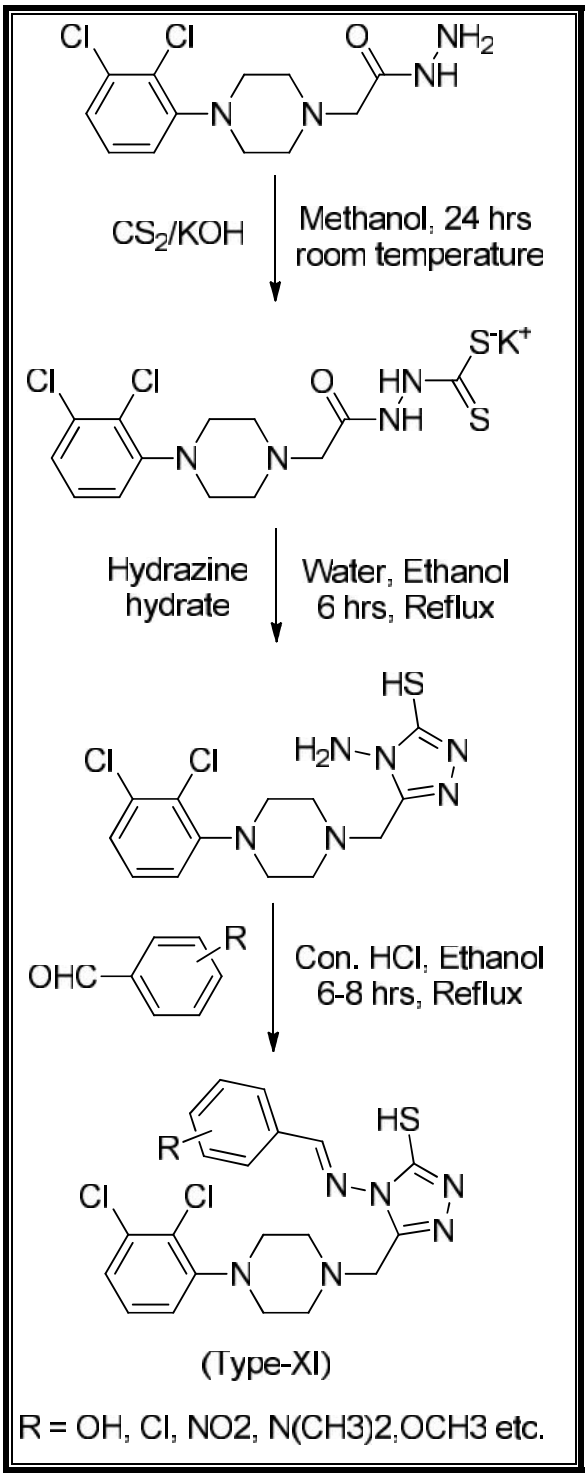
SYNTHESIS AND BIOLOGICAL SCREENING OF 4-((ARYLMETHYLIDINE) AMINO)-5-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-4*H*-1,2,4-TRIAZOLE-3-THIOL

1,2,4-Triazoles nucleus and their derivatives emerge rapidly with the advance of modern heterocyclic chemistry, promising a variety of medical applications such as antibacterial, antifungal, anticancer, antitumor, anticonvulsant, anti-inflammatory and analgesic properties. Schiff bases of 1,2,4-triazoles find diverse applications and extensive biological activity. Looking to this, schiff base derivative of type-XI have been synthesised by the condensation of 4-amino-5-((4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-4*H*-1,2,4-triazole-3-thiol with different substituted aromatic aldehyde.

The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

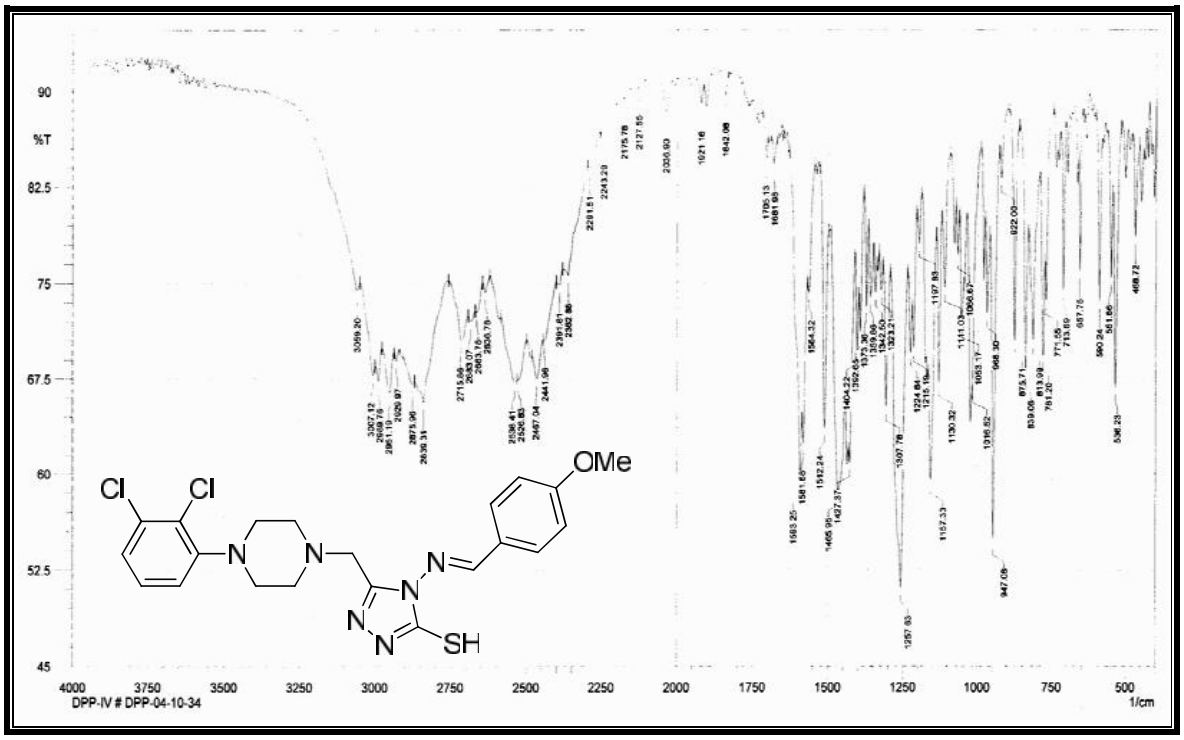
REACTION SCHEME



(Type-XI)

R = OH, Cl, NO2, N(CH3)2,OCH3 etc.

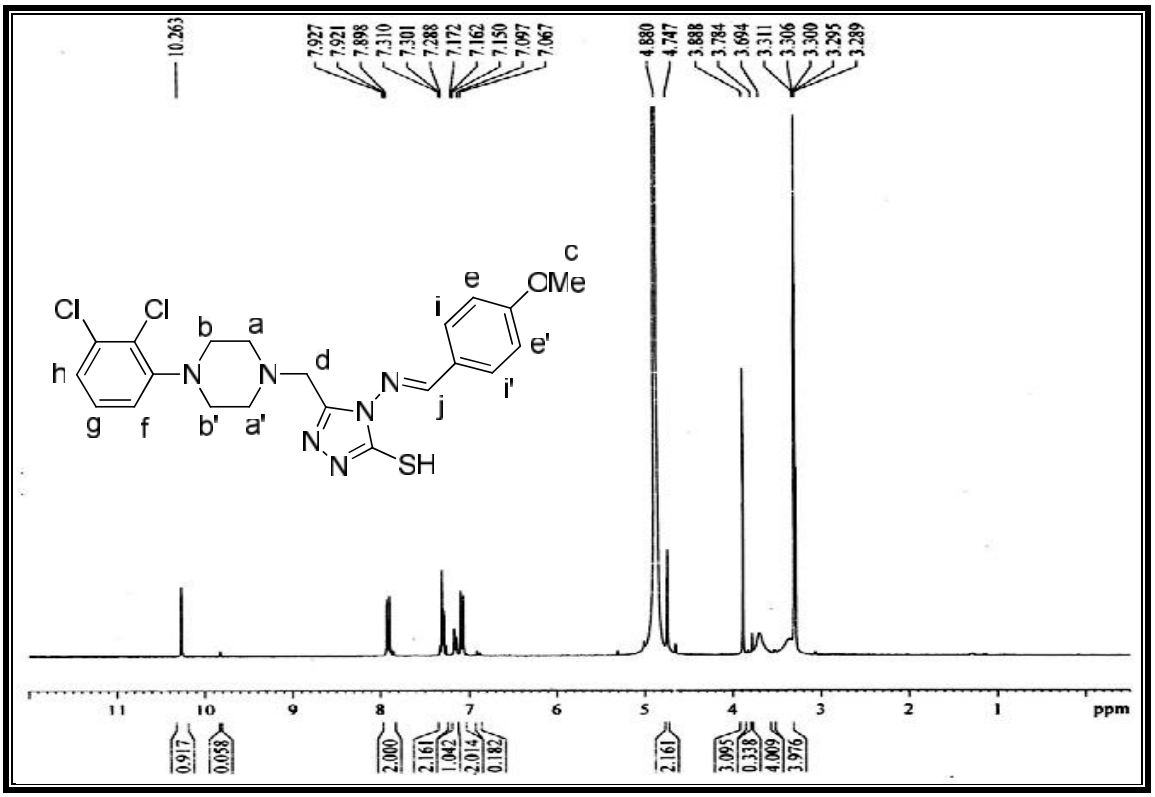
IR SPECTRUM OF 5-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-4-(((4-METHOXYPHENYL)METHYLIDENE)AMINO)-4*H*-1,2,4-TRIAZOLE-3-THIOL



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2951	2975-2920	57
	C-H str. (sym.)	2875	2880-2860	”
	C-H def. (asym.)	1465	1470-1435	”
	C-H def. (sym.)	1392	1395-1370	”
Aromatic	C-H str.	3059	3100-3000	”
	C=C	1512	1585-1480	”
	C-H i.p. def.	1130	1125-1090	”
	C-H o.o.p. def.	839	860-810	”
Triazole ring & Azomethine	N=C str.	1593	1650-1580	58
	C-N str.	1307	1350-1200	”
	N-N str.	1157	1220-1020	”
	-S-H str.	2538	2600-2550	
Ether (-OMe)	C-O-C	1224	1275-1200	57
Halide	C-Cl	813	850-650	”

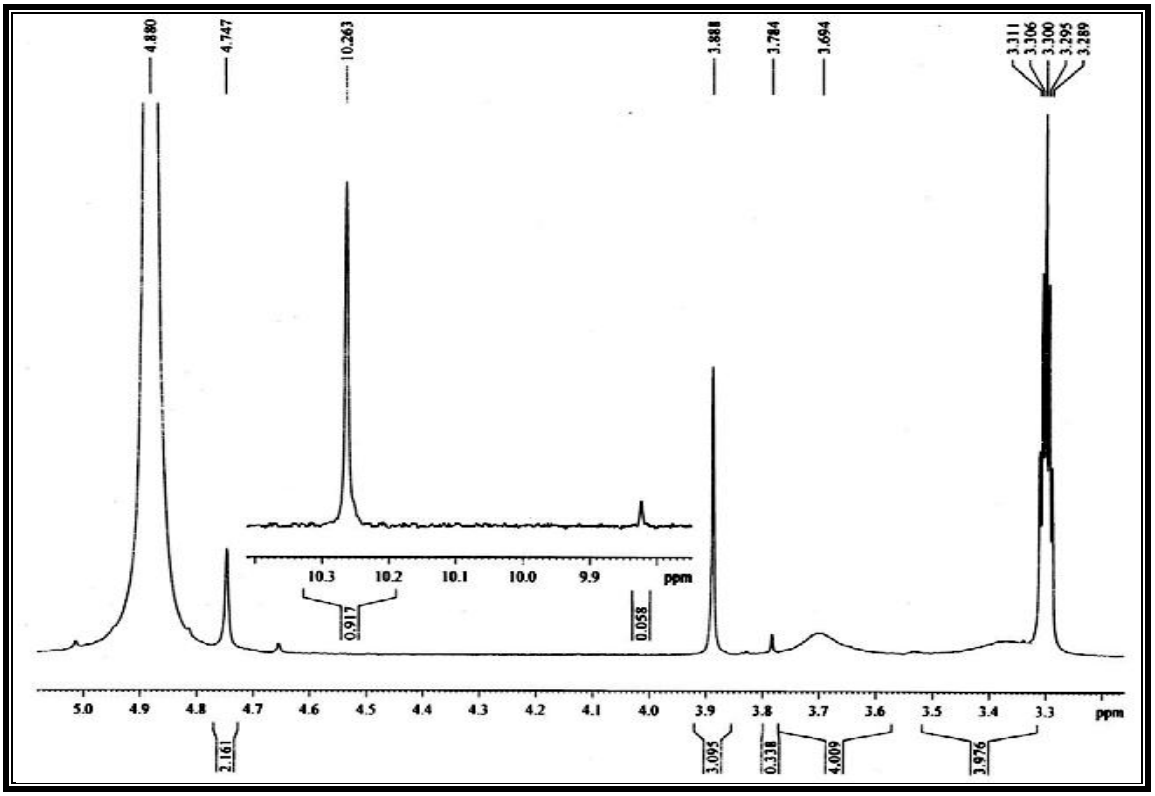
^{1}H-NMR SPECTRUM OF 5-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-4-(((4-METHOXYPHENYL)METHYLIDENE)AMINO)-4*H*-1,2,4-TRIAZOLE-3-THIOL

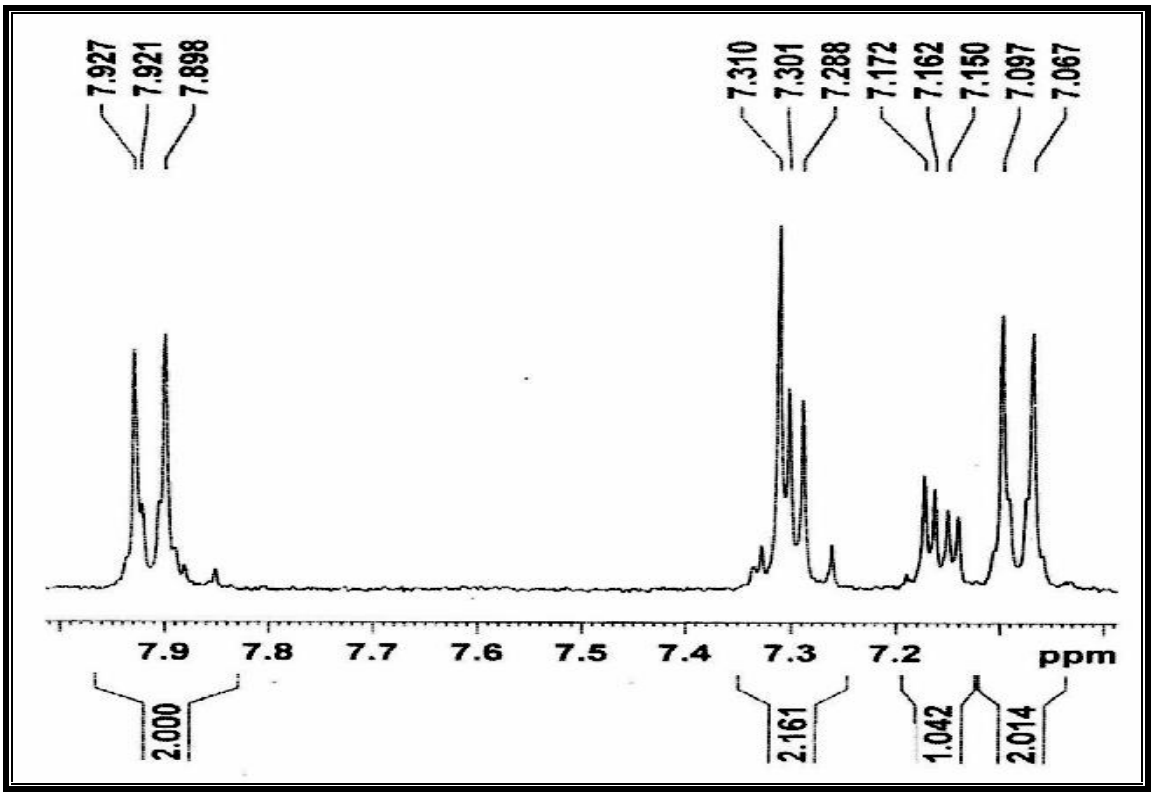


Internal Standard: TMS; Solvent: MeOD Instrument: BRUKER Spectrometer (300MHz)

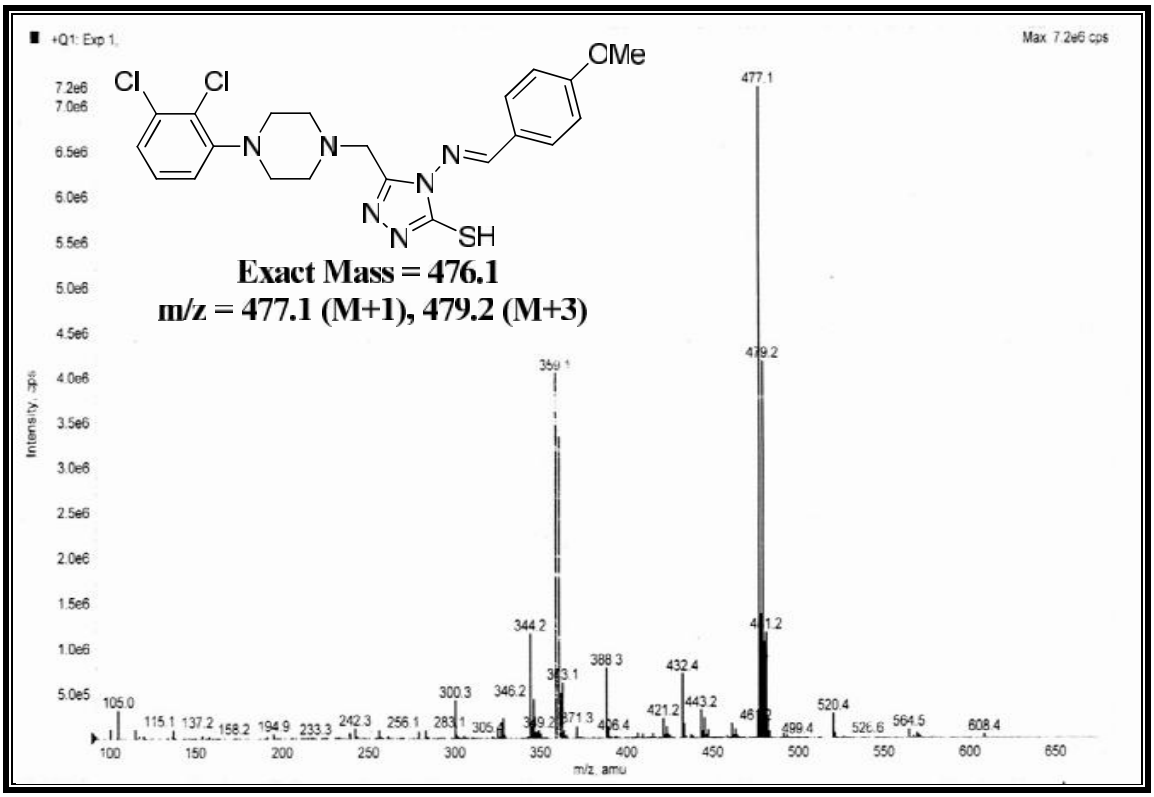
Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	3.37	4H	broad singlet	-C<u>H</u>$_2$ (a,a')	-
2	3.12-3.14	4H	broad singlet	-C<u>H</u>$_2$ (b,b')	-
3	3.88	3H	singlet	-OC<u>H</u>$_3$ (c)	-
4	4.74	2H	singlet	-N-C<u>H</u>$_2$ (d)	-
5	7.06-7.09	2H	doublet	Ar-H (e,e')	9.0
6	7.15-7.17	1H	double doublet	Ar-H (f)	3.0 & 6.6
7	7.28-7.31	2H	multiplet	Ar-H(g,h)	-
8	7.89-7.92	2H	doublet	Ar-H (i,i')	8.7
9	10.26	1H	singlet	-N=C<u>H</u> (j)	-

EXPANDED ^{1}H-NMR SPECTRUM

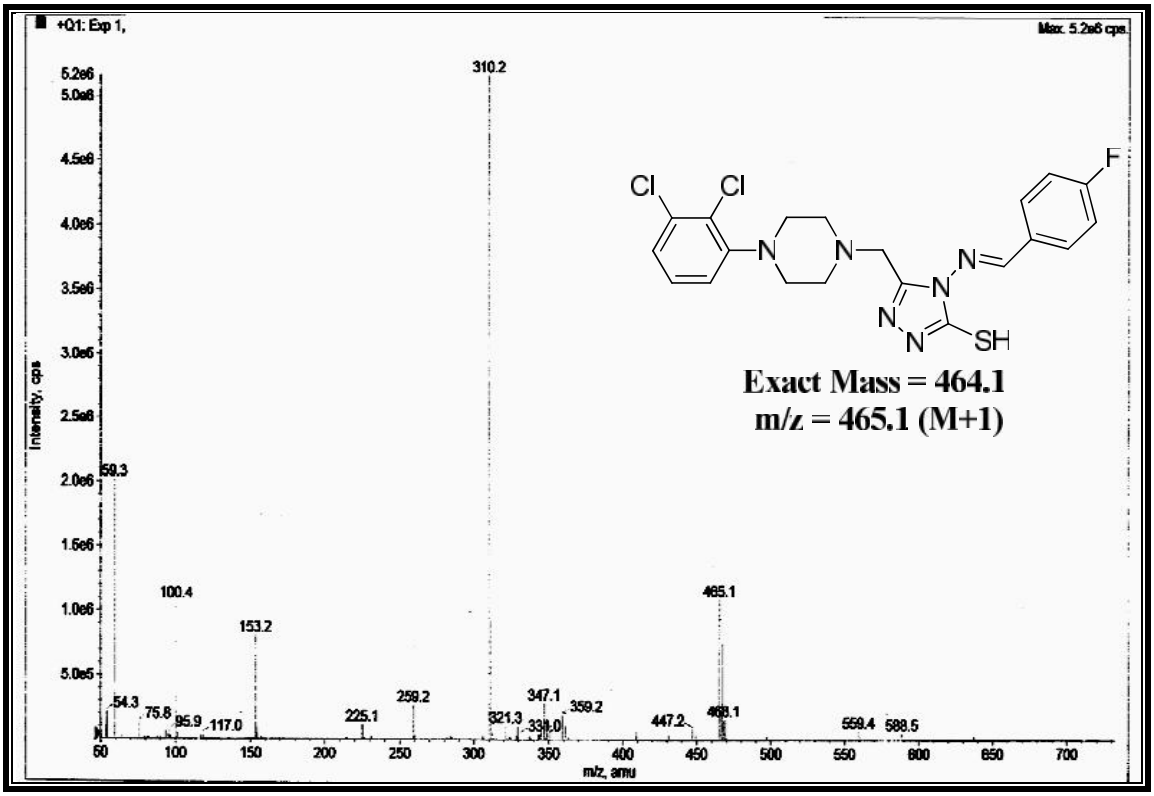




MASS SPECTRUM OF 5-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-4-(((4-METHOXYPHENYL)METHYLIDENE)AMINO)-4*H*-1,2,4-TRIAZOLE-3-THIOL



MASS SPECTRUM OF 5-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-4-(((4-FLUOROPHENYL)METHYLIDENE)AMINO)-4*H*-1,2,4-TRIAZOLE-3-THIOL



EXPERIMENTAL

SYNTHESIS AND BIOLOGICAL SCREENING OF 4-((ARYLMETHYLIDINE) AMINO)-5-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-4*H*-1,2,4-TRIAZOLE-3-THIOL

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. 70 % Ethyl acetate in hexane was used as a mobile phase.

[A] SYNTHESIS OF 2-(4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)ACETOHYDRAZIDE

See, Chapter-2, Part-A, Section-I, Experimental [B], page no. 72.

[B] SYNTHESIS OF POTASSIUM 2-(2-(4-(2,3-DICHLOROPHENYL) PIPERAZIN-1-YL)ACETYL)HYDRAZINECARBODITHIOATE

To the mixture of potassium hydroxide (8.40g, 0.15mol) and 2-(4-(2,3-dichlorophenyl)piperazin-1-yl)acetohydrazide (30.2g, 0.1mol) in ethanol (100 ml), carbon disulphide (11.4g, 0.15mol) was added. This mixture was stirred for 24 hrs at room temperature. It was then diluted with dry ether (400 ml) and thus the solid obtained was filtered and washed with ether and dried. There is no need to purify the salt for further reaction.

[C] SYNTHESIS OF 4-AMINO-5-((4-(2,3-DICHLOROPHENYL) PIPERAZIN-1-YL)METHYL)-4*H*-1,2,4-TRIAZOLE-3-THIOL

A suspension of the potassium salt (41.74g, 0.1mol), hydrazine hydrate (25 ml, 0.5mol), water (10 ml) and ethanol (50 ml) was refluxed with stirring for 8 hrs. The color of the reaction mixture changed to green, hydrogen sulfide was evolved and a homogeneous solution resulted. Dilute the solution with cold water (300 ml) and neutralized with glacial acetic acid, precipitated a white solid. The product was filtered, washed with cold water and crystallized from dioxane yield 60 %.

[D] SYNTHESIS OF 5-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL) METHYL)-4-(((4-METHOXYPHENYL)METHYLIDENE)AMINO)-4*H*-1,2,4-TRIAZOLE-3-THIOL

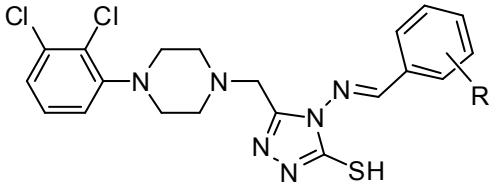
A mixture of 4-amino-5-((4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-4*H*-1,2,4-triazole-3-thiol (3.58 gm, 0.01 mol), 4-methoxybenzaldehyde (1.36 gm, 0.01 mol) and ethanol (20 ml) in presence of con. HCl (2 drops) was refluxed for 8 hrs. The contents were cooled and solid was filtered, dried and isolated product was recrystallized from ethanol. Yield: 81 %, M. P. 208-210 °C, ($C_{21}H_{22}C_{l2}N_6OS$; Required: C, 52.83; H, 4.64; N, 17.60 %; Found: C, 52.75; H, 4.56; N, 17.55 %).

Similarly, other 4-((arylmethylidine)amino)-5-((4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)-4*H*-1,2,4-triazole-3-thiol **(11a-j)** were prepared. The physical constants are recorded in **Table-11a,** Page no. 221.

[E] BIOLOGICAL SCREENING OF 4-((ARYLMETHYLIDINE)AMINO)-5-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-4*H*-1,2,4-TRIAZOLE-3-THIOL

Antimicrobial testing was carried out as described in Chapter-1**,** Section-I, Experimental [C], Page no. 37. The results obtained from antimicrobial testing are recorded in **Table-11b,** Page no. 222.

TABLE-11a: PHYSICAL CONSTANTS OF 4-((ARYLMETHYLIDINE)AMINO)-5-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-4*H*-1,2,4-TRIAZOLE-3-THIOL



Sr. No.	Substitution R	Molecular Formula/ Molecular Weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
11a	H	$C_{20}H_{20}Cl_2N_6S$ 447.38	193-196	86	53.59 53.41	4.51 4.46	18.78 18.69
11b	4-OMe	$C_{21}H_{22}Cl_2N_6OS$ 477.41	208-210	81	52.83 52.75	4.64 4.56	17.60 17.55
11c	4-F	$C_{20}H_{19}Cl_2FN_6S$ 465.37	218-220	89	51.62 51.38	4.12 4.04	18.06 17.97
11d	3-Cl	$C_{20}H_{19}Cl_3N_6S$ 481.83	184-186	82	49.85 49.70	3.97 3.89	17.44 17.35
11e	2,3-di Cl	$C_{20}H_{18}Cl_4N_6S$ 516.27	211-212	76	46.53 46.38	3.51 3.46	16.28 16.21
11f	4-$N(Me)_2$	$C_{22}H_{25}Cl_2N_7S$ 490.45	176-179	84	53.88 53.71	5.14 5.07	19.99 19.86
11g	3-OMe-4-OH	$C_{21}H_{22}Cl_2N_6O_2S$ 493.41	229-231	74	51.12 50.94	4.49 4.37	17.03 16.92
11h	4-NO_2	$C_{20}H_{19}Cl_2N_7O_2S$ 492.38	222-225	87	48.79 48.66	3.89 3.84	19.91 19.82
11i	3-NO_2	$C_{20}H_{19}Cl_2N_7O_2S$ 492.38	198-200	79	48.79 48.58	3.89 3.84	19.91 19.83
11j	4-OH	$C_{20}H_{20}Cl_2N_6OS$ 463.38	208-210	83	51.84 51.56	4.35 4.23	18.14 18.02

TABLE-11b: BIOLOGICAL SCREENING OF 4-((ARYLMETHYLIDINE) AMINO)-5-((4-(2,3-DICHLOROPHENYL)PIPERAZIN-1-YL)METHYL)-4*H*-1,2,4-TRIAZOLE-3-THIOL

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	11a	250	200	100	200	**250**	1000	1000
2	11b	250	250	200	**62.5**	**250**	1000	>1000
3	11c	**100**	200	100	200	500	250	250
4	11d	200	100	250	250	1000	>1000	>1000
5	11e	**100**	200	**50**	100	500	500	500
6	11f	500	500	500	250	>1000	>1000	>1000
7	11g	200	500	250	500	1000	500	500
8	11h	250	250	500	500	1000	1000	1000
9	11i	500	250	500	250	500	1000	>1000
10	11j	500	500	250	250	500	500	1000

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted triazole **11c** (R= 4-F) against *S.aureus,* **11e** (R= 2,3-di Cl) against *E-coli* and **11b** (R= 4-OMe) against *P.aeruginos* show excellent activity compared to ampicillin. While **11d** (R= 3-Cl) & **11g** (R= 3-OMe-4OH) against *S.aureus*, **11d** (R= 3-Cl) against *S.pyogenus,* **11a** (R= -H) & **11c** (R= 4-F) against *E-coli* and **11e** (R= 2,3-di Cl) against *P.aeruginos,* display moderate activity as compared to ampicillin. The remaining compounds demonstrate moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted triazoles **11a** (R= -H) & **11b** (R= 4-OMe) possess highly promisssing activity against *C.albicans* as compared to greseofulvin while **11c** (R= 4-F) display moderate activity against *A.niger* and *A.clavatus*. The remaining compounds show moderate to poor activity against all three bacterial species.

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Chapter-7

STUDIES ON ARYL AMIDE DERIVATIVES

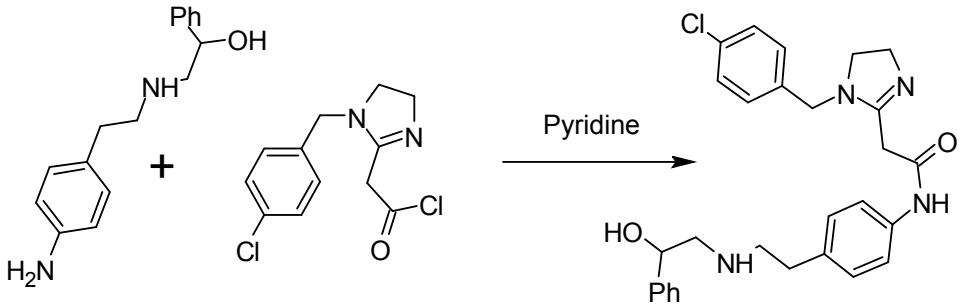
INTRODUCTION

The characteristic group present in the simple carboxylic amide is $CONH_2$. They are the acyl substitution products of ammonia. Many natural products are amides (-CO-NH-), urea (-NH-CO-NH-) and diamides (-CO-NH-CO-NH) derivatives of carbonic acid. The peptides and proteins are linear structure of cyclic polyamides. The alkaloids of pepper, piperidine and chavicine are N-substituted amides of unsaturated acid. N-isobutyl amides of certain highly unsaturated aliphatic acids occur in plants, shows insecticidal activity.[1] Amides derived from polyacetylenic acid have been isolated from certain fungi.[2]

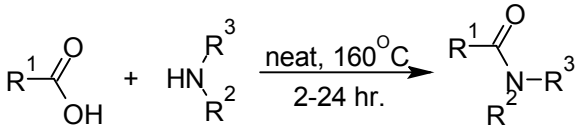
SYNTHETIC ASPECT

Various methods for the synthesis of aryl amides are described in literature.[3-9]

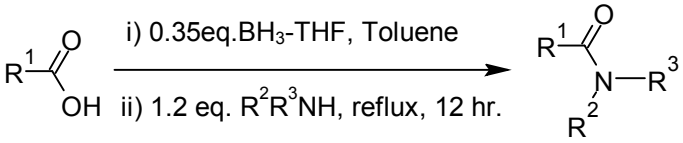
1. Marayama, Tatsuya, Suzuki Onda[10] have synthesized arylamide as under.



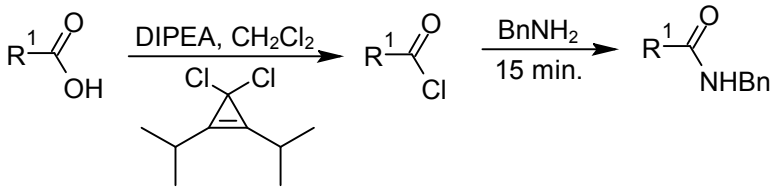
2. An effective protocol by thermal condensation of carboxylic acids with amines has been reported by L. J. Gooben et al.[11]



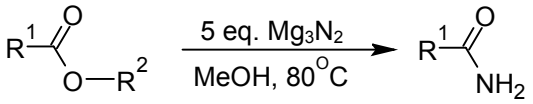
3. Z. Huang et al.[12] have prepared borane-tetrahydrofuran complex used to generate triacyloxyboranes, which can be effectively reacted with various nucleophiles (alkylamines, arylamines, hydrazides, alcohols, phenols) at reflux temperature in toluene to provide the corresponding amides.



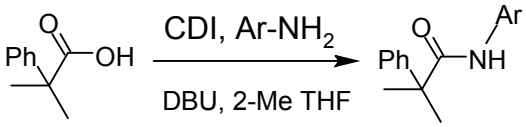
4. D. J. Hardee and coworkers[13] suggested that the conversion of carboxylic acids to their corresponding acid chlorides occurs rapidly in the presence a *tertiary* amine base and 3,3-dichlorocyclopropenes via aromatic cation-activated nucleophilic acyl substitution to give the corresponding amides.



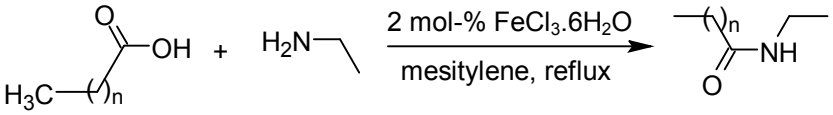
5. G. E. Veitch et al.[14] have used magnesium nitride as a convenient source of ammonia allows a direct transformation of esters to primary amides. Methyl, ethyl, isopropyl, and tert-butyl esters are converted to the corresponding carboxamides in good yields.



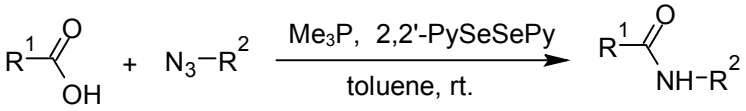
6. C. Larrive-Aboussafy et al.[15] have synthesized DBU catalyzed corresponding amides derivatives.



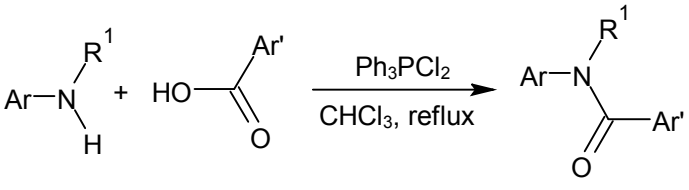
7. Multivalent metal salts, such as ferric chloride and sulfate, are active and versatile catalysts for the amidation of aliphatic fatty acids with long-chain aliphatic amines was reported by Y. Terada et al.[16]



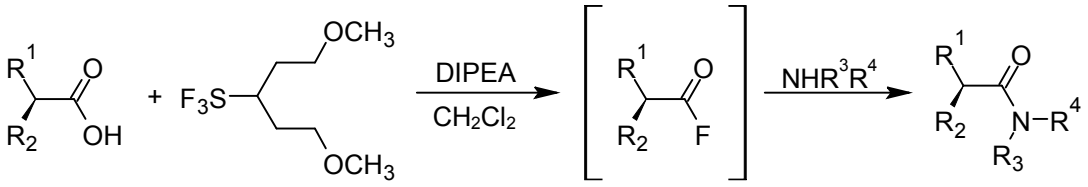
8. J. Bures et al.[17] have reported 2,2′-dipyridyl diselenide catalyzed direct reaction of carboxylic acids with azides and trimethylphosphine at room temperature.



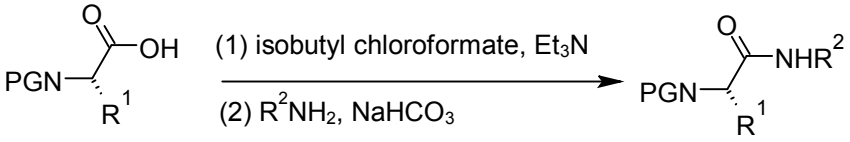
9. I. Azumaya et al.[18] have prepared various *tertiary* benzanilide derivatives in high yields from a broad range substituted benzoic acid and *N*-monoalkylated anilines using dichlorotriphenylphosphorane in chloroform.



10. Deoxo-Fluor is a versatile and mild reagent for acyl fluoride generation and subsequent one-pot amide coupling. J. M. White et al.[19] have reported the conversion of acids to amides and the use of deoxo-fluor as peptide-coupling reagent. Products were isolated after facile purification in good yields.



11. D. M. Shendage et al.[20] have prepared stereoconservative protection and deprotection method of amino and carboxyl groups includes the generation of *N*-phthaloyl *N′*-alkyl secondary amides from *N*-phthaloyl amino acids by using a mixedanhydridemethod.



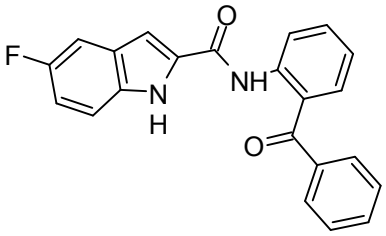
THERAPEUTIC IMPORTANCE

Amides, aryl amides and heterocyclic aryl amides showed various pharmacological activities. The biological activities of aryl amide derivatives have been reported as under.

1. Anticonvulsant[21]
2. Herbicidal[22]
3. Cardiotonic[23]
4. Antimicrobial[24,25]
5. Analgesic[26]
6. Antiulcer[27]
7. MAO inhibitor[28]
8. Anticancer[29,30]
9. Antiinflammatory[31]
10. Anti-HIV[32]
11. Sodium channel blockers[33,34]

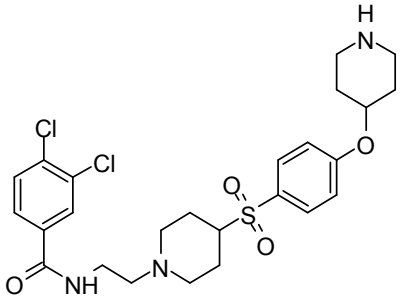
G. Shattat et al.[35] have synthesized and evaluated anti-hyperlipidemic activity of *N*-(benzoylphenyl)-5-fluoro-*1H*-indole-2-carboxamide (1) derivatives. H. B. Rubins and coworkers[36] have studied carboxamide derivatives as pharmacological mechanism of

fibrates, including bezafibrate, by the induction of lipoprotein lipase and reduction of apolipoprotein C-III synthesis leading to increased hydrolysis of triglycerides (TG). S. Olgen et al.[37] and G. Liu et al.[38] have studies the potential role for carboxamide derivatives as anti-allergics and antioxidants.

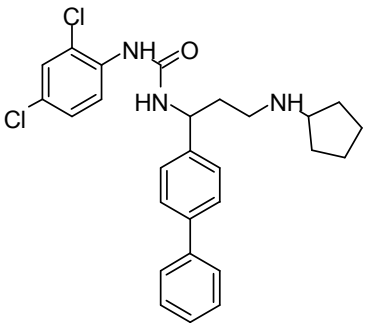


(1)

A. K. Mallams[39] has reported arylamide derivatives as antitumor agent. More over S. J. Laulloo et al[40] and J. Hazarika[41] have prepared some new biologically active arylamide derivatives and reported them as antimicrobial agents. Dhanak Dushyant et al.[42] have synthesized arylamide (2) useful as urotensin-II antagonist. E. J. Sanderson Philip et al.[43] (3) have synthesized aryl amides and studied their biological activity.



(2)



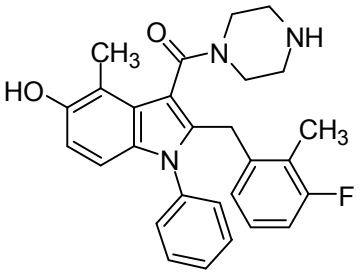
(3)

C. Kishor Kumar et al.[44] have synthesized some 3-oxoisoindoline-5-carboxamides and studied their antioxidant activity.

M. B. Anthony et al.[45] have reported aryl amide derivatives as antiulcer agents. J. E. Foster et al.[46] have synthesized some new amide derivatives as potent anticonvulsant. A. R. Mulik et al.[47] have studied some aryl amides shows antibiotic activity. G. Bridge et al.[48] have screened arylamides as anti-HIV agent. L. Bettineti and co-workers[49] have prepared arylamides as antibacterial agents. D. W. Hobbs et al.[50] have designed some amides and reported them as MCH-receptor antagonist.

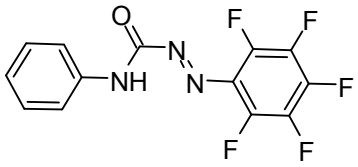
S. Henning et al.[51] have reported carboxamide derivatives (4), as renin inhibitors and renin-angiotensin modulators useful in the treatment of hypertension. C. Zhong et al.[52] have synthesized and reported antitumor activities of some carboxamide derivatives.

R. Aleksandra et al.[53] have documented rational design, synthesis, and potency of 1*H*-indole-5-carboxamide as potential fructose 1,6-bisphosphatase inhibitors.

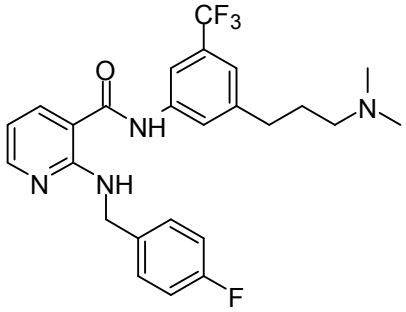


(4)

S. Tabuchi et al.[54] have screened arylamide derivatives as novel potent antagonist of human neuropeptide YY5 receptor. J. H. Chan[55] has reported substituted benzophenone-arylamide derivatives as inhibitor reverse transcriptase. L. Pieters et al.[56] have synthesized new diazene carboxamides (5) as anticancer agent. N. J. Anthony and co-workers[57] have investigated amides for anti-HIV activity. G. Chen et al.[58] have reported aryl amides (6) as antitumor agents.

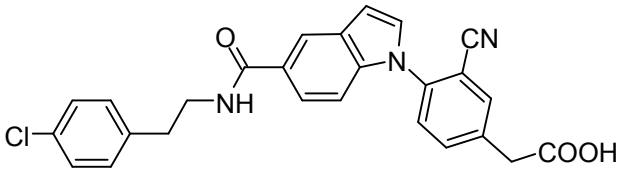


(5)



(6)

G. A. Doherty et al.[59] have synthesized indole-5-carboxamide derivatives (7) as DP2 receptor modulators for treating immunological diseases. Indole carboxamide derivatives applied as p38α-selective MAP kinase inhibitor which reduces tumor growth in mouse xenograft models of multiple myeloma and SD-282 reduces inflammation in a subchronic model by S. Medicherla et al.[60]

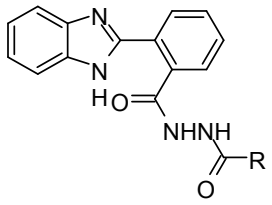


(7)

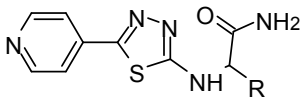
CONTRIBUTION FROM OUR LABORATORY

Some new arylamide derivatives bearing benzimidazol moiety (8) were assessed by H. H. Parekh et al.[61] and evaluated its antimicrobial activity. A. R. Parikh et al.[62-65]

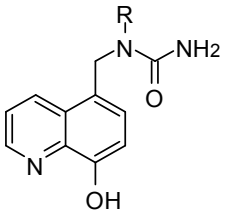
have synthesized new arylamide derivatives (9), (10) and reported them as antimicrobial agents.



(8)

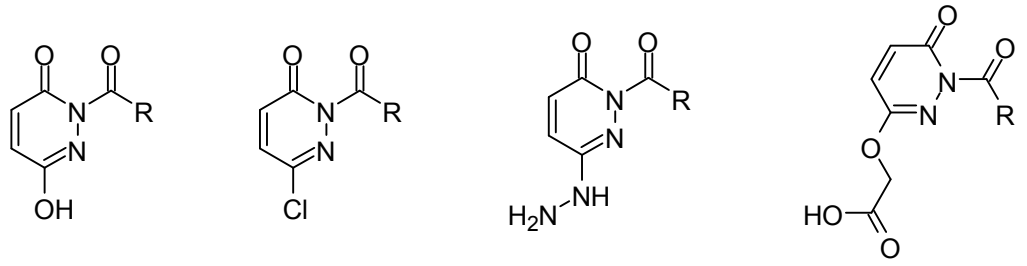


(9)



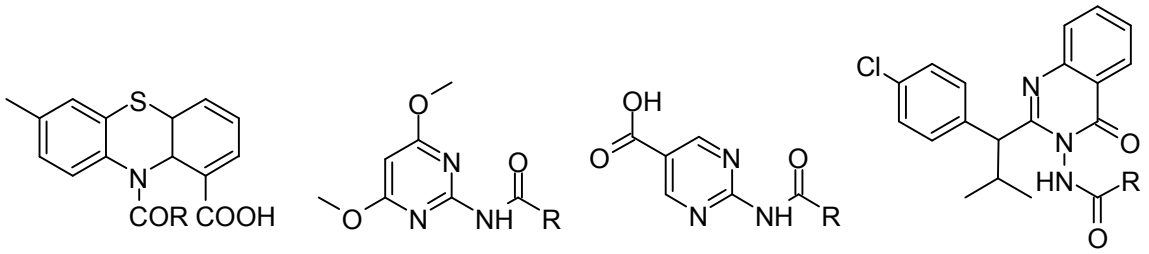
(10)

V. H. Shah et al.[66] have synthesized some new aryl amides (11) and evaluated their antimicrobial activity.



(11)

V. N. Patoliya et al.[67-70] have synthesized and evaluated antimicrobial activity of some aryl amide derivative (12). D. M. Purohit et al.[159] have reported new aryl amides derivatives having piperazine moiety as a antimicrobial agent.



(12)

Looking to the interesting properties of aryl amide derivatives, we have synthesized some aryl amides bearing a chromene moiety, which have been described as under.

SECTION-I: SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-(ARYLCARBONYL)-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

SECTION-I

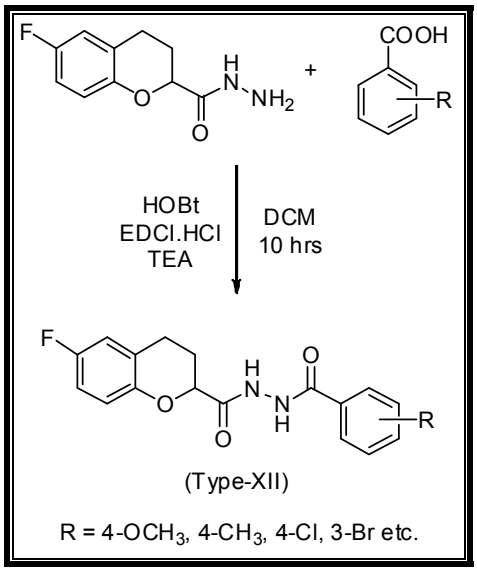
SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-(ARYLCARBONYL)-6-FLUORO- 3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

Arylamide derivatives showed different biological activity such as antihista manic, anti-inflammatory, anticonvulsant, antitubercular, antipyretic, analgesic, antiseptic etc. To getting better therapeutic agent and to evaluate its pharmacological profile, we have synthesize aryl amides of type-(XII) by the condensation of 6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide with different aromatic acid in the presence of *N*-[3-(dimethylamino)propyl]-*N*'-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxybenzotri-azole (HOBt) and triethylamine (TEA).

The constitution of the synthesized products have been characterized by using elemental analysis, IR & ^{1}H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their *in vitro* biological assay like antibacterial activity towards *Gram positive* and *Gram negative* bacterial strains and antifungal activity towards *A. niger, C. Albicans and A. Clavatus* at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

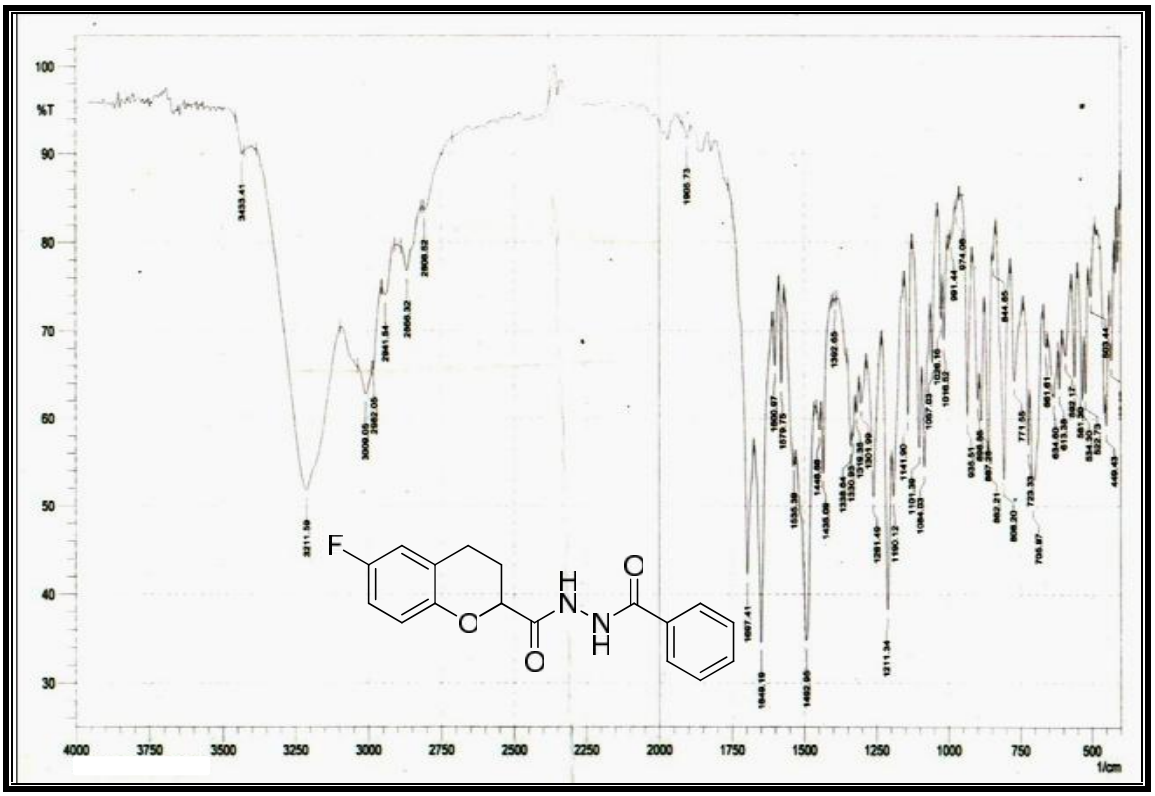
REACTION SCHEME



(Type-XII)

R = 4-OCH_3, 4-CH_3, 4-Cl, 3-Br etc.

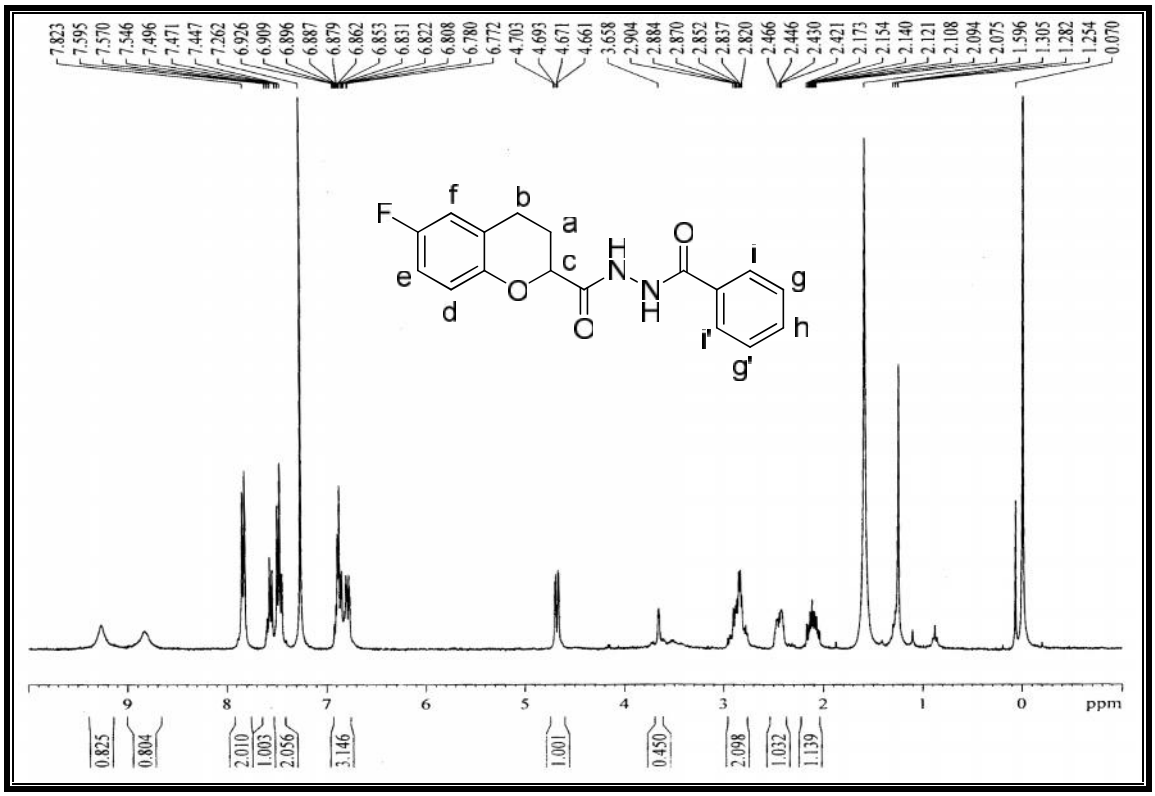
IR SPECTRUM OF 6-FLUORO-*N*'-(PHENYLCARBONYL)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm^{-1} (KBr disc.)

Type	Vibration Mode	Frequency cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2941	2975-2920	72
	C-H str. (sym.)	2866	2880-2860	”
	C-H def. (asym.)	1435	1470-1435	”
	C-H def. (sym.)	1338	1395-1370	”
Aromatic	C-H str.	3009	3100-3000	”
	C=Cstr.	1535 & 1492	1585-1480	”
	C-H i.p. def.	1089	1125-1090	”
Amide	C=O str.	1697 & 1649	1700-1650	”
	-NH str.	3211	3320-3140	”
	C-N str.	1101	1250-1020	
Ether	C-O-C	1261	1275-1200	”

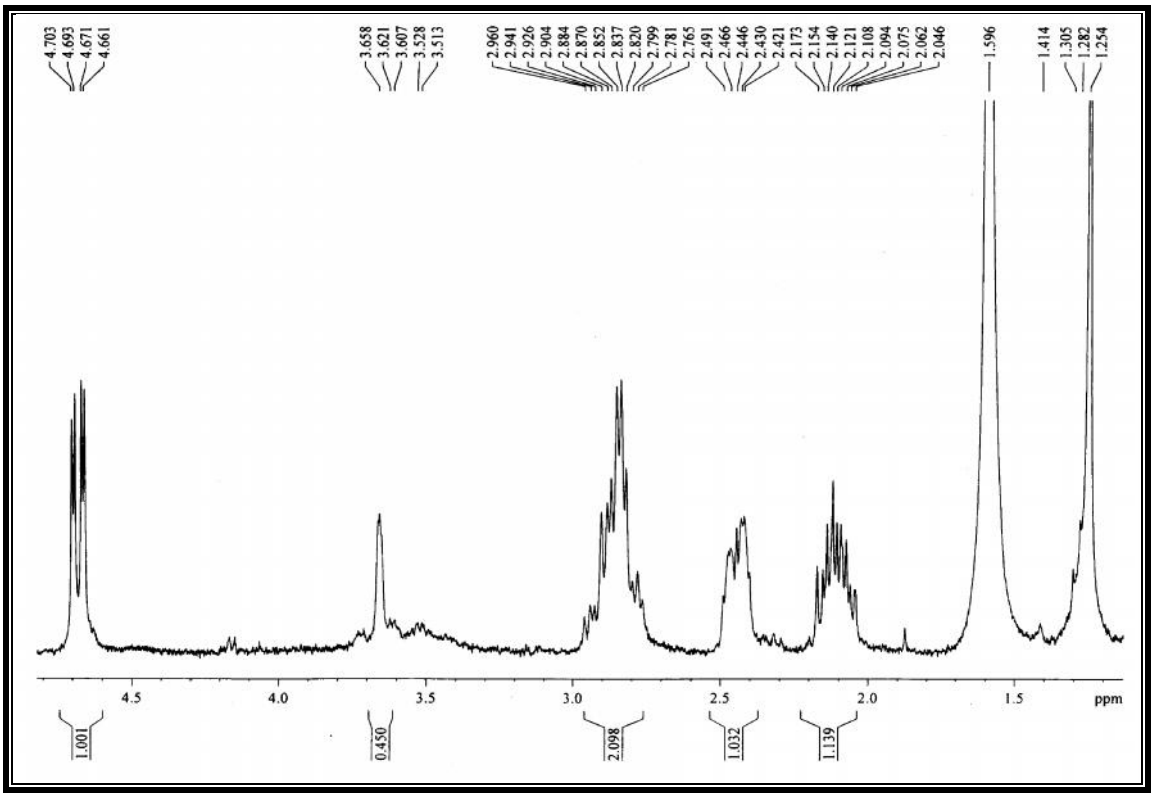
^{1}H-NMR SPECTRUM OF 6-FLUORO-*N*'-(PHENYLCARBONYL)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

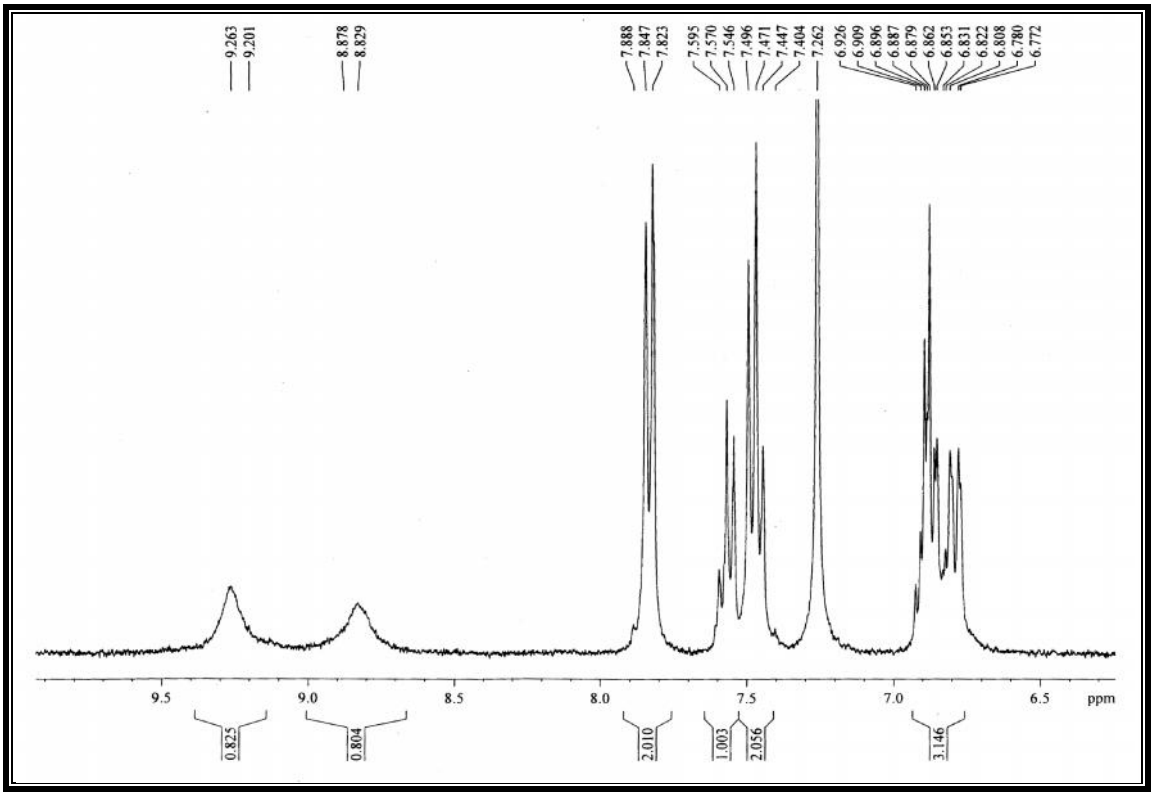


Internal Standard: TMS; Solvent: $CDCl_3$ Instrument: BRUKER Spectrometer (300MHz)

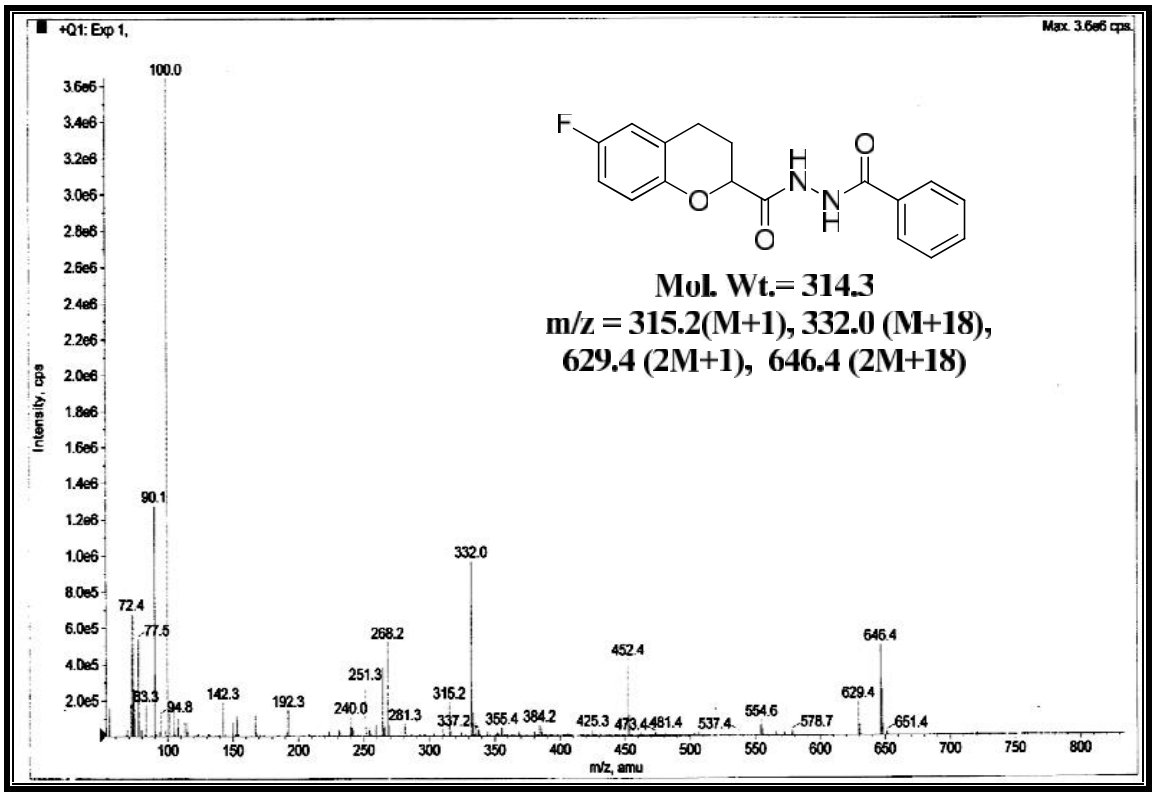
Sr. No.	Chemical Shift In δppm	Relative No. of Protons	Multiplicity	Inference	J Value in HZ
1	2.04-2.17	1H	multiplet	-C<u>H</u>$_2$ (a)	-
2	2.42-2.46	1H	multiplet	-C<u>H</u>$_2$ (a)	-
3	2.82-2.90	2H	multiplet	-C<u>H</u>$_2$ (b)	-
4	4.66-4.70	1H	double doublet	-C<u>H</u> (c)	3.0 & 9.6
5	6.77-6.92	3H	multiplet	Ar-H (d,e,f)	-
6	7.44-7.49	2H	triplet	Ar-H(g,g')	-
7	7.54-7.59	1H	triplet	Ar-H(h)	-
8	7.82-7.84	2H	doublet	Ar-H(i,i')	7.2
9	8.82	1H	singlet	-CO-N<u>H</u>	-
10	9.26	1H	singlet	-CO-N<u>H</u>	-

EXPANDED ^{1}H-NMR SPECTRUM

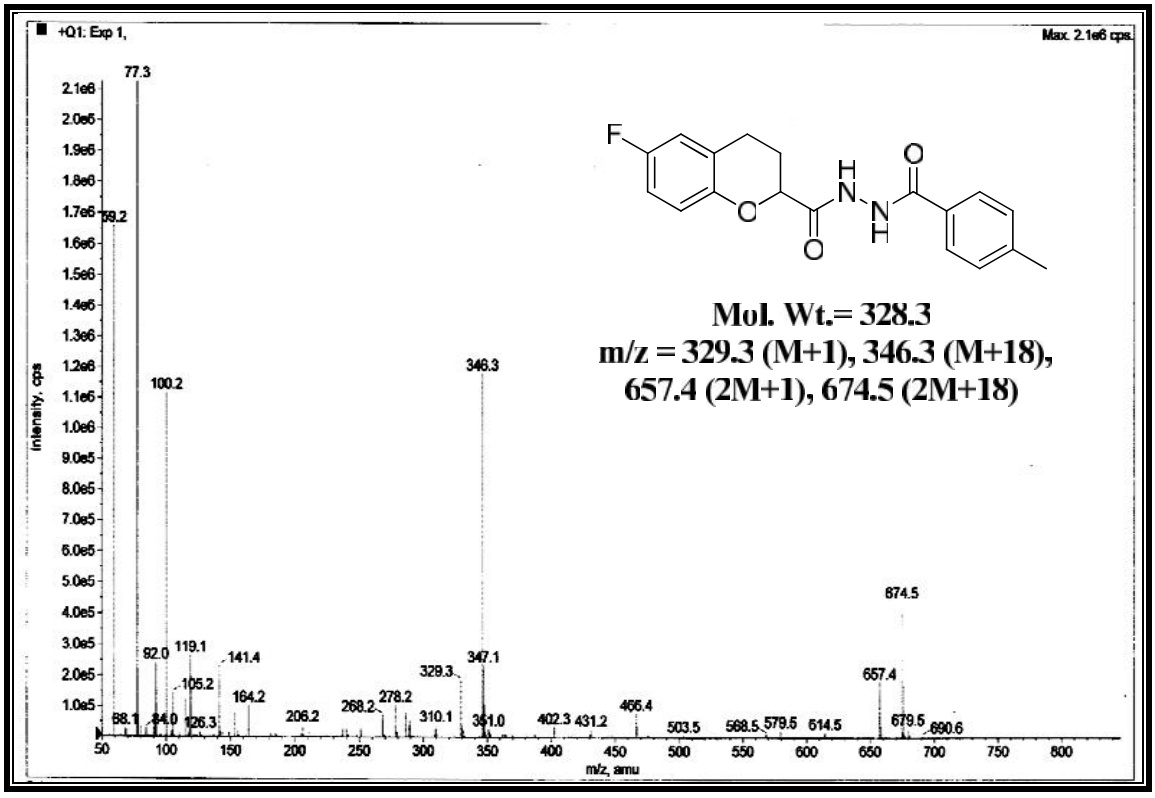




MASS SPECTRUM OF 6-FLUORO-*N*'-(PHENYLCARBONYL)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



MASS SPECTRUM OF 6-FLUORO-*N*'-((4-METHYLPHENYL)CARBONYL)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



EXPERIMENTAL

SYNTHESIS AND BIOLOGICAL SCREENING OF *N*'-(ARYLCARBONYL)-6-FLUORO- 3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F_{254} coated aluminum sheet (Merck prepared plates) as stationary phase. 10 % Methanol in chloroforrm was used as a mobile phase.

[A] SYNTHESIS OF 6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

See, Chapter-2, Part-B, Section-I, Experimental [B], Page no. 93.

[B] SYNTHESIS OF 6-FLUORO-*N*'-(PHENYLCARBONYL)-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

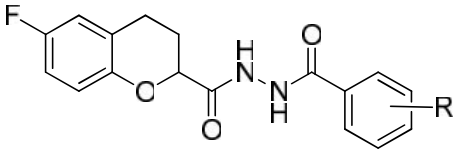
To the stirred solution of benzoic acid (1.22 g, 0.01 mol) in dry DCM (20 ml), HOBt (*N*-hydroxybenzotrizole) (1.53 g, 0.01 mol) and EDCI (*N,N'*-dicyclohexylcarbodi-imide) (2.87 g, 0.015) was added at 0 ^{o}C. The obtained solution was stirred for 15 minute at 0 ^{o}C. To this solution 6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide (2.10 g, 0.01 mol) was added portionwise, then after 2 minute TEA (2.08 ml 0.015 mol) was added. The reaction mixture was stirred for 10 hour at room temperature (monitored by TLC). The solvent was distillled out, residue was poured into water. The product was extracted with ethylacetate (20 ml × 3), and the combined organic layers were washed with water followed by brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum and the residue was purified by column chromatography on silica gel (mesh size: 60-120 (eluent: 2 % methanol in DCM) to obtain pure product. Yield: 79 %, M. P. 203-204 °C, ($C_{17}H_{15}FN_2O_3$; Required: C, 64.96; H, 4.81; N, 8.91 %; Found: C, 64.61; H, 4.72; N, 8.85 %).

Similarly, other *N*'-(arylcarbonyl)-6-fluoro-3,4-dihydro-2*H*-chromene-2-carbohydrazide were **(12a-j)** prepared. The physical constants are recorded in **Table-12a**, Page no. 241.

[C] BIOLOGICAL SCREENING OF *N*'-(ARYLCARBONYL)-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

Antimicrobial testing was carried out as described in Chapter-1**,** Section-I, Experimental [C], Page no. 37. The results obtained from antimicrobial testing are recorded in **Table-12b,** Page no. 242.

TABLE-12a: PHYSICAL CONSTANTS OF *N'*-(ARYLCARBONYL)-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE



Sr. No.	Substitution R	Molecular Formula/ Molecular Weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
12a	H	$C_{17}H_{15}FN_2O_3$ 314.31	203-204	79	64.96 64.61	4.84 4.72	8.91 8.85
12b	4-OMe	$C_{18}H_{17}FN_2O_4$ 344.34	185-187	73	62.79 62.48	4.98 4.91	8.14 8.01
12c	4-CH_3	$C_{18}H_{17}FN_2O_3$ 328.34	197-198	76	65.84 65.68	5.22 5.17	8.53 8.39
12d	4-NO_2	$C_{17}H_{14}FN_3O_5$ 359.31	226-228	69	56.83 56.66	3.93 3.88	11.69 11.57
12e	4-F	$C_{17}H_{14}F_2N_2O_3$ 332.30	177-179	65	61.44 61.32	4.25 4.16	8.43 8.38
12f	4-Cl	$C_{17}H_{14}ClFN_2O_3$ 348.76	192-194	72	58.55 58.31	4.05 3.98	8.03 7.94
12g	3-Br	$C_{17}H_{14}BrFN_2O_3$ 393.21	183-185	67	51.93 51.78	3.59 3.48	7.12 7.02
12h	3-OMe	$C_{18}H_{17}FN_2O_4$ 344.34	205-208	64	62.79 62.57	4.98 4.93	8.14 8.05
12i	3- CH_3	$C_{18}H_{17}FN_2O_3$ 328.34	217-219	68	65.84 65.68	5.22 5.14	8.53 8.47
12j	2-NO_2-5-Cl	$C_{17}H_{13}ClFN_3O_5$ 393.75	199-201	59	51.86 51.72	3.33 3.29	10.67 10.53

TABLE 12b: BIOLOGICAL SCREENING OF *N*'-(ARYLCARBONYL)-6-FLUORO-3,4-DIHYDRO-2*H*-CHROMENE-2-CARBOHYDRAZIDE

Sr. No.	Code	Antibacterial Activity				Antifungal activity		
		Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
		Gram +*ve* Bacteria		Gram –*ve* Bacteria				
		S.aureus	*S.pyogenus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
1	12a	500	250	250	250	**250**	1000	500
2	12b	**200**	500	500	500	1000	>1000	500
3	12c	250	**50**	200	250	1000	>1000	>1000
4	12d	500	250	500	500	500	1000	>1000
5	12e	250	100	200	250	1000	>1000	1000
6	12f	**200**	200	**62.5**	100	500	200	250
7	12g	500	500	250	250	500	1000	>1000
8	12h	250	250	250	500	1000	500	500
9	12i	500	250	500	250	500	1000	1000
10	12j	500	250	100	250	**250**	500	1000

MINIMAL INHIBITION CONCENTRATION

Standard Drugs	*S.aureus*	*S.pyogenus*	*E.coli*	*P.aeruginosa*
	(microgramme/ml)			
Gentamycin	0.25	0.5	0.05	1
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	50	50	25	25
Norfloxacin	10	10	10	10

MINIMAL FUNGICIDAL CONCENTRATION

Standard Drugs	*C.Albicans*	*A.Niger*	*A.Clavatus*
	(microgramme/ml)		
Nystatin	100	100	100
Greseofulvin	500	100	100

ANTIBACTERIAL ACTIVITY:

From screening results, substituted aryl amide **12b** (R= 4-OMe) & **12f** (R= 4-Cl) against *S.aureus,* **12c** (R= 4-Me) against *S.pyogenus* and **12f** (R= 4-Cl) against *E-coli* exhibit very good activity compared to ampicillin. While **12c** (R= 4-Me) & **12e** (R= 4-F) against *S.aureus*, **12e** (R= 4-F) against *S.pyogenus,* **12j** (R= 2-NO_2-5-Cl) against *E-coli* and **12f** (R= 4-Cl) against *P.aeruginos,* show moderate activity as compared to ampicillin. The remaining compounds demonstrate moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted aryl amide **12a** (R= -H) & **12j** (R= 2-NO_2-5-Cl) display excellent activity against *C.albicans* as compared to greseofulvin while **12f** (R= 4-Cl) exhibit moderate activity against *A.niger* and *A.clavatus*. The remaining compounds show moderate to poor activity against all three bacterial species.

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Publications

List of Publication

1. **Synthesis, Characterization and Anti-microbial Evaluation of Some Novel 1,3,4-Oxadiazoles Containing Piperazine Moiety**

   Nirav K. Joshi[*], Dinesh S. Kundariya and Jaysukhlal M. Parmar; *International Journal of Chemtech Research* , 4(4), 1503-1508 (2012).

2. **Mass Spectral Fragmentation Modes of Pyrimidine derivatives**

   J. M. Parmar[*] and **N. K. Joshi**; *International Journal of Chemtech Research,* 4(3), 1247-1254 (2012).

3. **Synthesis and Structure-Activity Relationship of potent, Selective and Orally Active Anthranilamide-Based Factor Xa Inhibitors: Application of Weakly Basic Sulfoximine Group as Novel S4 Binding Element**

   Vrajesh Pandya, Mukul Jain, Ganes Chakrabarti, Hitesh Soni, Bhavesh Parmar, Balaji Chaugule, Jigar Patel, Tushar Jarag, Jignesh Joshi, **Nirav Joshi**, Akshyaya Rath, Vishal Unadkat, Bhavesh Sharma, Haresh Ajani, Jeevan Kumar, Kalapatapu V.V.M. Sairam, Harilal Patel, Pankaj Patel; *European journal of Medicinal Chemistry* (In Press, Available online from 15th October 2012, Accepted Manuscript-Manuscript no.: EJMECH-D-12-00908)

4. **Discovery of inhibitors of plasminogen activator inhibitor-1: Structure-activity study of 5-nitro 2-phenoxybenzoic acid derivatives**

   Vrajesh Pandya, Mukul Jain, Ganes Chakrabarti, Hitesh Soni, Bhavesh Parmar, Balaji Chaugule, Jigar Patel, Jignesh Joshi, **Nirav Joshi**, Akshyaya Rath, Mehul Raviya, Mubeen Shaikh, Kalapatapu VVM Sairam, Harilal Patel, Pankaj Patel; *Bioorganic and Medicinal Chemistry Letters*, 21(19), 5701-5706 (2011).

5. **Synthesis, Characterization and Pharmacological Evaluation of Some Novel Schiff Bases Containing 1*H*-pyrazolo[3,4-b]pyridine Moiety**

   D. S. Kundariya[*], B. M. Bheshdadia, **N. K. Joshi** and P. K. Patel; *International Journal of Chemtech Research,* 1(3), 238-243 (2011).

6. **Synthesis towards 4,6-Disubstituted Pyrimidines via Chalcone Derivatives and their Biological Evaluation**

   D. S. Kundariya[*], **N. K. Joshi** and P. K. Godhaviya; *Research Journal of Pharmaceutical, Biological and Chemical Sciences* (Accepted article-Acceptance Ref.No.: RJPBCS/2010-1640)